THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 138 MARCH 1, 1943 No. 4

THE EFFECT OF SYMPATHOMIMETIC AMINES UPON THE OUTPUT OF RESPIRATORY TRACT FLUID IN RABBITS

ELDON M. BOYD, SHIRLEY JACKSON AND ALICE RONAN

From the Department of Pharmacology, Queen's University, Kingston, Canada

Received for publication October 8, 1942

Perry and Boyd (1) have reported that stimulation of the cervical vagus nerve and injections of pilocarpine nitrate will produce a marked increase in the output of respiratory tract fluid (R.T.F.) collected from a cannula in the trachea of urethanized rabbits and cats. In the present communication corresponding data will be presented upon the effect of faradic electrical stimulation of the cervical sympathetic trunk and upon the effect of adrenaline hydrochloride and other sympathomimetic amines.

Most of the work was performed upon healthy, adult rabbits with a few experiments upon guinea pigs. The animals were anesthetized with urethane and a side arm cannula ligated into the trachea after the method of Perry and Boyd (1). Subsequently, several modifications were made from the method of Perry and Boyd. The side arm of the tracheal cannula was connected with a heat-insulated, double right angle bent glass tube which, in turn, connected with a glass tube of wide bore housed in a box wherein the temperature was kept constant at 38°C. by an electric bulb connected in series with a De Khotinsky thermoregulator and condenser and a small electric fan to maintain a circulation of air. The wide bore glass tube passed directly through this box and inside the wide bore tube were plugs of cotton lying upon a wire tray and kept continuously moist by water, dripping at a rate regulated by a Hoffmann screw clamp upon a piece of rubber tubing which connected to a reservoir Mariotte bottle of water placed on the top of the heating box. This arrangement substituted for the nasal passage, which had been eliminated, and warmed and moistened the air which the rabbits inhaled. R.T.F. which drained from the trachea was collected in a graduated, heat-insulated, 15 ml. centrifuge tube attached to the distal end of the tracheal cannula.

The animals were fastened upon their backs or bellies to especially built operating tables in which the copper-covered, heated top could be raised from a base upon a hinge located at the head of the table and thus hold the animal, head downward, at any satisfactory angle which permitted a slight decline in the tube which received the R.T.F. The actual posture of the body has been

shown by Boyd and Ronan (2) to be without influence upon the volume of R.T.F. collected.

Thus assembled, R.T.F. was collected over a period of 3 hours. The volume of R.T.F. was noted at intervals of one hour, divided by the weight of the animal in kilograms and multiplied by 24 to give a figure which indicated the output of R.T.F. in milliliters per kilogram body weight per 24 hours. During the first hour the output was erratic but by the end of the third hour it had settled down to a fairly steady rate in most animals. At the end of the third hour adrenaline hydrochloride, dissolved in the B.P. vehicle, was injected subcutaneously in various doses and in a volume of 1 ml. per kilogram body weight. Controls received at the same time an equivalent volume of adrenaline vehicle only. A summary of the results has been given in table 1 in which the output

TABLE 1

The effect of various sympathomimetic drugs upon the output of respiratory tract fluid

ANIMAL	NO. OF ANIMALS	DRUG	DOSE (MGM./KILO)	OUTPUT OF R.T.F.-ML./KILO/24 HRS.					
				Hrs. before drug		Hrs. after drug			
				2	1	1	2	3	4
Guinea pig	13	Controls		1.7	1.8	1.6	2.2	2.1	2.0
Guinea pig	11	Adrenaline	0.1 to 1	2.5	2.9	4.3	2.7	2.8	2.8
Rabbit	18	Controls		3.3	3.4	3.8	3.0	3.2	3.4
Rabbit	7	Adrenaline	1	1.7	2.5	2.0	3.3	2.6	2.5
Rabbit	13	Adrenaline	5	2.7	2.7	2.8	2.2	3.2	1.7
Rabbit	14	Ephedrine	10 to 500	3.1	4.1	4.2	3.5	3.3	3.4
Rabbit	8	Neo-synephrine	10 to 500	2.8	4.4	4.5	3.4	2.8	2.4
Rabbit	8	Amphetamine	1	2.2	2.2	2.4	2.3	1.9	1.5
Rabbit	12	Amphetamine	10	3.5	4.1	4.5	5.5	4.7	4.4
Rabbit	9	Privine	1	2.6	3.0	4.1	3.9	1.9	1.9
Rabbit	19	Privine	10	3.1	3.3	4.1	3.8	3.5	2.3
Rabbit	14	Sympathetic stimulation		2.9	2.6	3.2	2.1	2.5	2.1

of R.T.F. has been given for the two hours immediately preceding the injection of adrenaline hydrochloride or vehicle and for four hours after.

Adrenaline hydrochloride was given in doses of 1 and 5 mgm. per kilogram body weight. As shown in table 1, the injection of this drug had no effect upon the output of R.T.F. A few doses of 10 mgm. per kilogram were tried but proved lethal in most rabbits. Corresponding doses given to guinea pigs gave an increase in the mean output one hour after injection which was due to two exceptionally high values; statistical calculations failed to reveal any significant effect of the drug.

Ephedrine hydrochloride was injected in a range of doses from 10 to 500 mgm. per kilogram body weight. As with adrenaline hydrochloride, there were no significant changes in the output of R.T.F.

Neosynephrine hydrochloride, N.N.R., was also injected, in doses similar

to those of ephedrine hydrochloride. Likewise it had no effect upon the amount of R.T.F. drained from the trachea of rabbits.

Amphetamine (Benzedrine) sulphate, N.N.R., was injected subcutaneously, in doses of 1 and 10 mgm. per kilogram body weight. Larger doses of 50 mgm. and over were tried but proved lethal to most rabbits. The dose of 1 mgm. per kilogram had no effect upon the output of R.T.F. Following the injection of 10 mgm. per kilogram, amphetamine increased the mean output of R.T.F. over a period of several hours. The results suggested that amphetamine might have some small effect upon the volume of R.T.F. excreted, an effect which, in view of the results with the previous sympathomimetic amines, was probably related to the stimulant effect of amphetamine upon the central nervous system rather than to its local sympathomimetic action which latter, in any event, is considerably different from that of adrenaline (3).

Another sympathomimetic substance investigated was Privine or 2-(naphthyl-1-methyl) imidazoline hydrochloride, a new derivative of histamine with an adrenaline-like action upon arterial muscle (4). It was injected in doses of 1 and 10 mgm. per kilogram body weight. There was some increase in the mean output of R.T.F. following the administration of this drug, but this was slight and not statistically significant.

Thus none of these sympathomimetic drugs, with the possible exception of amphetamine, had any significant effect upon the volume output of R.T.F. A corresponding result was obtained by faradic electrical stimulation of the cervical sympathetic nerve trunk.

The cervical sympathetic trunk lying in the carotid sheath was dissected carefully from the vagus nerve and the carotid artery for a length of about 1 cm. with the rabbit under urethane anesthesia. The nerve was raised slightly from nearby tissues from which it was then separated by a thin sheet of condom rubber and from time to time the nerve was moistened with saline solutions. Silver electrodes were applied to the nerve which was stimulated every 5 seconds for a period of 1 second with a faradic electrical current from an induction coil connected in series with a Brodie cut-out key. From the point of stimulation, afferent fibres pass downward to the inferior cervical ganglion from which arise the efferent fibres to the lung. While it could not be stated with certainty that impulses actually reached the acinar glands of the bronchial mucosa by stimulation of the sympathetic at the point described, nevertheless this technique was considered preferable to stimulation at or below the inferior cervical ganglion which involved artificial respiration, an undesirable complication in experiments designed to measure the output of R.T.F. Thus assembled, R.T.F. was collected from rabbits over a period of 2 to 3 hours, until a steady rate had been obtained. Then the cervical sympathetic nerve was stimulated for a period of from 1 to 3 hours and its effect upon the output of R.T.F. noted. After this followed a period of no nerve stimulation for a corresponding length of time and these two procedures were repeated, as time permitted, and alternated in different animals.

A total of 18 such experiments was performed. The mean output of R.T.F.

during sympathetic nerve stimulation was 3.5 ml. per kilogram body weight per 24 hours and the mean output during the periods of no sympathetic stimulation was 3.3 ml. per kilogram body weight per 24 hours.

These results indicated that stimulation of the cervical sympathetic nerve by this method had no effect upon the output of R.T.F. It seemed desirable to find if continuous stimulation over a longer period of time might be with effect. Consequently, rabbits were arranged as before and the cervical sympathetic trunk stimulated at 5 second intervals over a period of up to 12 hours. Controls were treated in a similar manner but given no faradic stimulation. There were 12 control rabbits and 14 rabbits with electrical stimulation of the cervical sympathetic trunk. The mean hourly output of R.T.F. in each group was calculated and plotted and the two curves were almost identical. The mean output of 75 hourly readings upon the controls was within 4 per cent of the mean output of 98 readings upon the cervical stimulated rabbits. For comparison with the output in rabbits receiving the sympathomimetic drugs, the means over corresponding hours have been included in table 1. These data indicated that stimulation of the cervical sympathetic trunk at the point indicated and with a faradic electrical current had no effect upon the output of R.T.F. These results are in line with those obtained from the administration of sympathomimetic drugs.

SUMMARY

The subcutaneous injection of a range of doses of the sympathomimetic drugs adrenaline, ephedrine, neosynephrine, amphetamine and privine was without effect upon the volume output of respiratory tract fluid drained from the trachea of urethanized rabbits, with the possible exception of large doses of amphetamine which may have slightly increased the output. Similarly, faradic electrical stimulation of the cervical sympathetic trunk had no effect upon the output of R.T.F.

Acknowledgment. The authors wish to acknowledge with thanks the receipt of a grant in aid of this research from the Ciba Company Limited of Montreal obtained through the courtesy of Mr. E. R. Angehrn.

REFERENCES

(1) Perry, W. F. and E. M. Boyd. J. Pharmacol. and Exper. Therap. **73:** 65, 1941.
(2) Boyd, E. M. and R. K. Ronan. This Journal **135:** 383, 1942.
(3) Boyd, E. M. and W. F. Connell. Am. J. Med. Sci. **194:** 678, 1937.
(4) Meier, R. and R. Müller. Schweiz. Med. Wchnschr. **71:** 554, 1941.

PLASMA PROTEINS (ALBUMIN AND GLOBULIN) AND RED CELL VOLUME FOLLOWING A SINGLE SEVERE NON-FATAL HEMORRHAGE

ROBERT ELMAN, CARL E. LISCHER AND HARRIET WOLF DAVEY

From the Department of Surgery, Washington University and Barnes Hospital, Saint Louis, Missouri

(Aided by a grant from the Commonwealth Fund)

Received for publication October 9, 1942

In a previous communication (1) in this Journal, observations were reported on the changes in the plasma proteins and in the red cell volume following a single severe hemorrhage from which the dogs recovered. Immediately after the bleeding the blood was replaced by the same volume of Ringer's solution, most of which rapidly left the circulation, a level being reached in about one hour. The observations were continued for only 6 hours thereafter, and showed that in this second period the hypoproteinemia produced was only slightly affected, but that there was evidence of an increase in the plasma volume and in the total plasma proteins.

In the present communication these observations are carried further, changes being observed in a larger series of experiments for 24 hours after the hemorrhage and in a smaller series of experiments up to 7 days after the hemorrhage, both with and without replacement.

PREVIOUS WORK. Earlier observations on the effect of severe hemorrhage on the plasma proteins were described in the previous paper (1). Since then a number of additional studies have been made. Ebert, Stead and Gibson (2) studied 6 human donors following a bleeding of 760 to 1220 cc. After the initial fall they found an increase of plasma volume (determined by the dye method) approximately equal to the volume of red cells removed. The concentration of plasma protein (determined by calculation from the specific gravity as measured by the falling drop method) fell and then rose only slightly for several days. Notable was the observation that the hematocrit value fell for 70 hours and paralleled the increase in plasma volume. In contrast to these findings are those of Wallace and Sharpey-Shaffer (3) who also studied donors after a loss of 500 to 1150 cc. of blood. Plasma protein (determined by Nesslerization) showed little change in practically all of the 28 cases; even the hemoglobin showed little change but did fall, reaching a maximum between 3 and 90 hours with an average of 32 hours.

In dogs, Calvin (4) bled 25 per cent of the calculated blood volume without fatality and studied the changes in plasma volume, albumin and globulin for 4 hours. Proteins were determined by a Kjeldahl method. The plasma volume rose due to the inflow of fluid containing protein which was assumed to be primarily albumin, inasmuch as the albumin-globulin ratio increased. Ebert, Stead, Warren and Watts, also in dogs (5) studied the spontaneous plasma protein replacement following moderate (2 to 3.5 per cent of body weight) as con-

trasted with much more severe (repeated) hemorrhage, and found that the process was impaired in the latter group. Fine, Fischman and Frank (6) also studied the effects of severe hemorrhage in dogs both with and without anesthesia; plasma proteins were determined by calculation from the specific gravity as measured by the falling drop method. Only the change at 4 hours after hemorrhage was measured and a variable but incomplete return of the total circulating protein was observed on experiments in which no parenteral fluid was given. Price, Hanlon, Longmier and Metcalf (7) bled 15 dogs under nembutal anesthesia a total of about 3.4 per cent of their body weights, all animals succumbing apparently in about 24 hours. They found that the change in the total circulating plasma protein could be accounted for by the amount removed. However, the concentration did fall slightly due presumably to a shift of protein-free fluid into the blood stream. Plasma volume fell and there was therefore little change in the hematocrit. Plasma proteins were calculated from the specific gravity of the plasma by the falling drop technic. Brandhendler et al. (8), in cats, found a fall in serum protein as high as 79 per cent in a series of severe hemorrhages, the return to normal requiring 6 days. In horses they found that 10 days were required for the correction of the hypoproteinemia following a severe bleeding. In 7 rabbits, Volinets (9) found after a hemorrhage of 25 per cent of the estimated blood volume that the albumin and globulin following bleeding returned toward normal within 24 hours and were complete in 10 days, though the albumin returned faster than the globulin.

EXPERIMENTAL PROCEDURES. Three groups of experiments were carried out. In the first group of 25 dogs, the standard hemorrhage as previously described (1) was carried out. Bleeding was conducted under local anesthesia by exposing the femoral artery which was cannulated and 35 cc. per kilogram of body weight of blood removed. Immediately the same volume of Ringer's solution was replaced through the same cannula.

In the second group, 12 dogs were bled the same amount and observed for 7 days. In 6 of these experiments, Ringer's solution was used as replacement, whereas in the remaining 6 a red cell suspension in Ringer's solution was used, constituting therefore a single plasmapheresis.

In the third group of 8 dogs, no fluid at all was replaced. The amount of blood removed was somewhat larger; in half it was 40, in the others 45 cc. per kilogram of body weight. All recovered.

The entire bleeding procedure rarely required more than a few minutes. Although a fall of blood pressure and moderate shock did occur, there were no fatalities with the exception of 2 animals in the last group in which no fluid was replaced. These experiments were discarded. All observations, therefore, were made on animals who recovered spontaneously from the experimental procedure. Previous to bleeding, no food had been ingested for 24 hours. After the hemorrhage, water was allowed ad libitum for 24 hours, after which a normal kennel ration was provided.

Samples of blood were obtained from the jugular vein, heparinized and immediately centrifuged at 3000 R.P.M. at 30 minutes to determine the red cell

volume; the supernatant plasma was then analyzed for total nitrogen and fractionated for albumin and globulin after subtraction of the non-protein nitrogen, which was determined with Nessler's reagent. For nitrogen determinations the titrimetric micro-Kjeldahl procedure described by Sobel, Yuska and Cohen (10) was used. Fractionation was carried out by the method described by Campbell and Hanna (11).

EXPERIMENTAL FINDINGS. In all experiments the results were calculated as a percentage change, using the initial value as 100. In the first group of 25 dogs, observations were made at 1 hour, 7 hours and 24 hours after the hemorrhage and plotted as a scatter diagram in figures 1 and 2, each point representing

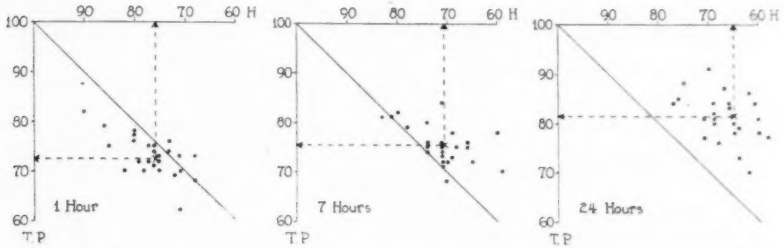

Fig. 1. Relation between red cell volume and concentration of total protein. Scatter diagram of 25 bleeding experiments described as group I in the text; each point represents one experiment. The average initial value shown as 100 per cent was, for the total protein (T.P.), 6.28 grams per cent, for the hematocrit (H) 48.8 per cent. The 45° line represents the theoretical distribution if hemodilution occurred with a protein free fluid, i.e., the red cells and the total protein would be affected equally. The crosses and arrows represent the arithmetic means and make it easier to follow the trend between 1, 7 and 24 hours.

Note the progressive fall in the average hematocrit value, indicating increasing hemodilution, most marked at 1 hour. The greater fall in protein as compared with hematocrit at 1 hour probably indicates the addition of red cells to the diluting fluid during this period. Note also (after the fall at 1 hr.) the increase of the total protein at 7 hours and 24 hours, indicating that a protein-containing fluid was responsible for hemodilution during these latter periods.

one experiment. In figure 1, the relationship between the total protein and the hematocrit value is shown. It is clear from a study of this figure that the red cell volume falls rapidly one hour after the hemorrhage and continues during the entire period of observation. This is also true of the concentration of the total protein during the first hour. However, after the first hour the fall in the total protein was replaced by a rise at 7 hours, which is continued at 24 hours. In figure 2, the relationship between the albumin and globulin indicates that the fall in globulin is slightly less pronounced than albumin at the first hour but that the increase in the concentration of total protein at 7 hours is due almost entirely to an increase in albumin fraction, confirming the findings of Calvin (4) as already mentioned. At 24 hours the changes in albumin and globulin are about the same. These changes are also shown in figure 4, which represents the mean of these values in the form of a graph.

In the second series of 12 experiments represented in figure 3, a picture is obtained of the changes occurring during one week after hemorrhage. It is obvious that the fall in the red cell volume continues for 72 hours, after which it is replaced by a slight rise at 7 days. The albumin fraction on the contrary, as shown also in figure 2, begins to increase after the precipitate fall at 1 hour; this increase is slowed at 72 hours and even at 7 days, the original concentration of albumin falls short of being reached by about 10 per cent. In contrast is the rapid return of the globulin fraction which reaches its normal level between the 24 and 72 hour specimens, continuing its increase so that at one week it has exceeded the original concentration by about 15 per cent.

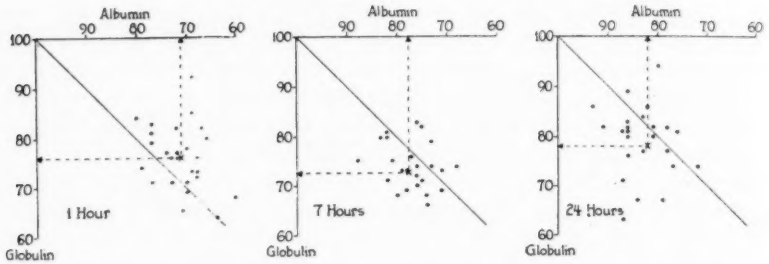

Fig. 2. Relation between concentration of albumin and globulin. Scatter diagram of the 25 bleeding experiments described in the text as group I. Each point represents one experiment. The average initial value represented as 100 per cent was, for the globulin, 2.5 grams per cent, for the albumin 3.68 grams per cent. The 45° line represents the theoretical distribution if the albumin and globulin behaved the same during the hemodilution. The crosses and arrows represent the arithmetic means and make it easier to follow the trend between 1, 7 and 24 hours; these means together with those of hematocrits are plotted as a curve in figure 4.

Note the slightly greater fall in albumin than globulin at 1 hour, indicating the addition of more globulin (fibrinogen?) than albumin to the diluting fluid at this time. At 7 hours, however, note the pronounced shift indicating the addition of relatively more albumin than globulin as shown by the increased concentration of this fraction in contrast to a further fall in the globulin. At 24 hours, however, the change in the albumin and globulin fractions is about the same, indicating a shift in the opposite direction, which, as shown in figure 3 and discussed in the text, is the beginning of the globulin regeneration which far outstrips the albumin from this point on.

Of special interest are the findings in the 6 experiments of group 2, in which plasmapheresis was carried out. As will be noted by consulting figure 3, the 1 hour specimen, of course, showed no fall in hematocrit, inasmuch as approximately the same volume of red cells was replaced as was removed. Nevertheless, in 24 hours the hematocrit value dropped more rapidly than in the animals in which the replacement consisted entirely of Ringer's solution. From this point on the curve of red cell volume parallels exactly the curve in the latter experiments. The steeper fall in the red cell volume between the first and 24 hour period is obviously due to a greater hemodilution and undoubtedly explains the difference in the behavior of the albumin and globulin fractions during

this period as compared with the bleeding in which Ringer's solution alone was replaced. Thus the albumin fell after plasmapheresis, in contrast to the rise in the experiments in which Ringer's solution alone was replaced. The change in the globulin showed a similar effect, i.e., its rise was less pronounced than in the animals in which Ringer's solution was replaced. After the 24 hour sample,

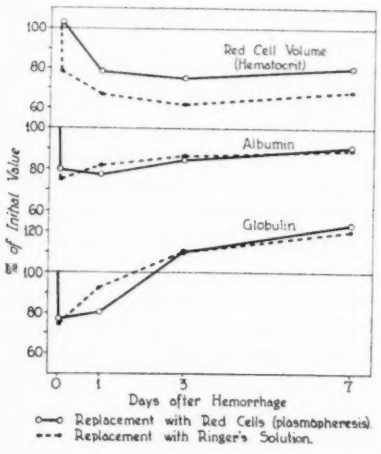

Fig. 3

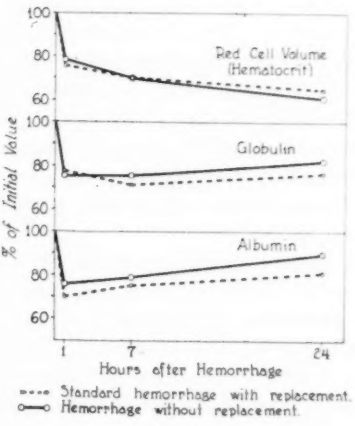

Fig. 4

Fig. 3. Relationships during one week. The two series of curves represented above were each plotted from the average values obtained in 6 bleeding experiments described in the text as group II.

Note the pronounced fall in the red cell volume at 24 hours which is continued more slowly at 72 hours and replaced by a slight rise at 7 days. Note also (after 24 hrs.) the relatively slow regeneration of albumin as compared with the rapid regeneration of globulin. Note also the general similarity in the curve when plasmapheresis was performed. For discussion of the difference in the plasmapheresis curve before 24 hours see text.

Fig. 4. The effect of replacement. The two series of curves represented above were plotted from averaged values. The broken line was made from the means shown in figures 1 and 2, i.e., the 25 standard bleeding experiments with replacement of Ringer's solution, described in the text as group I; the solid line was made from data obtained in 8 bleeding experiments without replacement described in the text as group III.

Note the similarity in the two curves, indicating the essential nature of the compensatory changes after hemorrhage regardless of whether or not the loss is replaced with Ringer's solution.

however, the changes in the albumin and globulin were similar in the two groups of experiments.

The third group of experiments was designed to determine just what part the replacement of Ringer's solution played in the hematocrit and serum protein curves. As can be seen by examining figure 4, very little difference was observed. To be sure, these animals were bled 4 and 4.5 per cent of their body weights, in contrast to the 3.5 per cent of the first group. Nevertheless, the fall in the

hematocrit value as well as in the albumin and globulin concentration was quite similar. Not recorded herein are the changes in this third group of experiments between the time of bleeding and the first hour; they were gradual and progressive, unlike the changes which occurred when blood was replaced with Ringer's solution. The immediate effect of replacement with Ringer's, as already mentioned, was a precipitate fall in hematocrit and plasma proteins, returning to a level in one hour, as the injected fluid left the circulation; the details were reported in our previous communication (1).

COMMENT. Two features of the present experiments should be emphasized. First, although the hemorrhage was severe, compensation was adequate and fatalities did not occur; in contrast, many experiments by others were associated with a fatal outcome. Second, a general anesthetic was not used. As is well known, barbiturates cause a dilatation of the spleen and a fall in the red cell volume; they also interfere with hemodilution after hemorrhage (12). Moreover, with general anesthesia, a smaller bleeding results in death in a shorter period of time (6).

Inferences from the present findings depend upon the assumption that the fall in the red cell volume measures the degree of plasma volume increase, i.e., hemodilution. This assumption seems justified by many considerations, including the following observations. In actual measurements of plasma volume with the dye method (2), the hematocrit changes reflected rather well the changes in the fluid volume of the blood. Further evidence by the same authors (13) showed a similar correlation between plasma volume and hematocrit value. Using red cells tagged with radioactive iron, a remarkable constancy was found (14) in total blood volume in dogs because "as the red cell circulating volume increases, there is a corresponding drop in the plasma volume."

Using the fall in the hematocrit value as an indication of plasma volume increase (hemodilution), it seems clear that fluid enters the blood stream after a severe non-fatal hemorrhage for as long as 72 hours, the rate being very pronounced in the first hour, less in the next 24 hours, and very slight thereafter. This phenomenon has been observed in the human (15, 2), the same period of maximum dilution (70 hrs.) occurring (2) as in the present experiments. From the fact that rapid dilution immediately followed plasmapheresis, it may be inferred that the hemodilution is not merely a phenomenon designed to replace the volume of the removed red cells; according to Starling's hypothesis, hemodilution actually may be due to a persistent fall in capillary pressure, induced by the hemorrhage.

The character of the fluid entering the blood immediately after the hemorrhage is probably protein-free, inasmuch as the concentration of protein at one hour is almost the same as the fall in red cell volume (fig. 1). The changes at 7 and 24 hours, however, indicate the entrance of fluid containing protein, whose fractions, albumin and globulin, each behave differently, particularly after the first hour. Between the first and seventh hours, it seems clear that the fluid entering the blood stream contains more albumin than globulin, inasmuch as the

concentration of albumin increases, whereas that of globulin falls (fig. 2). However, between the 7th and 24th hours, both increase. After the 24 hour period, the globulin increases much more rapidly, the albumin lagging far behind and never reaching its normal level, even after 7 days, while the globulin fraction exceeds its normal level at this period by 15 per cent (fig. 3). This difference in the behavior of albumin and globulin may be explained by assuming that the early addition of albumin to the diluting fluid is probably mobilized directly from the liver, whereas the subsequent but more pronounced addition of globulin would seem to be an actual regeneration.

On the basis of the present findings and of those of others, a biochemical approach to the problem of shock following hemorrhage seems justified. Using the term compensation to imply recovery from the effects of a severe hemorrhage, one might say that a hemorrhage is compensated when hemodilution is sufficient to restore and maintain blood volume adequately to support the circulation. Hemodilution is insufficient in maintaining blood volume only because of the low protein content of the diluting fluid.

The protein content of the fluid which restores blood volume is of decisive importance because it contributes the colloidal osmotic pressure of the blood upon which circulation and fluid interchange depend. The fact that the diluting fluid becomes poor in albumin is significant because albumin is responsible for 85 per cent of the blood's colloidal osmotic pressure.

The defect in the compensatory mechanisms would seem, therefore, to lie in the inability of the body to correct the hypoalbuminemia induced by the hemorrhage. Expressed in other words, fatalities following severe loss of blood are due to the inability of the body to restore blood volume with fluid containing sufficient albumin. This biochemical approach to the problem of shock in hemorrhage places the emphasis on an acute protein deficiency, and suggests the need for means to increase the output of albumin by the liver in order to obviate the necessity of supplying exogenous protein. Experiments along this line are now in progress.

SUMMARY

1. Hemodilution as shown by a falling red cell volume was a uniform finding in 45 non-fatal severe hemorrhages carried out without anesthesia with or without replacement of Ringer's solution or red cells. The most pronounced hemodilution occurred in the first hour, but continued at a decreased rate for 72 hours.

2. The fall in plasma proteins accompanying spontaneous hemodilution is greatest 1 hour after the bleeding. Correction of the low albumin fraction begins rapidly in the first 6 hours thereafter, but then slows, being still incomplete at 7 days. In contrast the globulin fraction continues to fall at 7 hours, but rapidly increases to its initial value between 24 and 72 hours.

3. The concept of acute protein deficiency is offered as a biochemical explanation of the problem of fatal (uncompensated) hemorrhage; i.e., the inability of the body to supply sufficient albumin during hemodilution.

REFERENCES

(1) ELMAN, R. This Journal **128:** 332, 1940.
(2) EBERT, R. V., E. A. STEAD AND J. G. GIBSON. Arch. Int. Med. **68:** 1578, 1941.
(3) WALLACE, J. AND E. P. SHARPEY-SCHAFER. Lancet **2:** 393, 1941.
(4) CALVIN, D. B. J. Lab. and Clin. Med. **26:** 1144, 1941.
(5) EBERT, R. V., E. A. STEAD, J. V. WARREN AND W. E. WATTS. This Journal **136:** 299, 1942.
(6) FINE, J., J. FISCHMANN AND H. A. FRANK. Surgery **12:** 1, 1942.
(7) PRICE, P. B., C. R. HANLON, W. P. LONGMIER AND W. METCALF. Bull. Johns Hopkins Hosp. **69:** 327, 1941.
(8) BRANDHENDLER, W. S. ET AL. Bull. de Biol. et de Med. Exper. **8:** 326, 1939.
(9) VOLINETS, I. J. Medical (U.S.S.R. Kiev) **8:** 790, 1938.
(10) SOBEL, A. E., H. YUSKA AND J. COHEN. J. Biol. Chem. **118:** 443, 1937.
(11) CAMPBELL, W. R. AND M. I. HANNA. J. Biol. Chem. **119:** 15, 1937.
(12) WEINER, D. O., R. ELMAN AND W. H. COLE. Proc. Soc. Exper. Biol. and Med. **32:** 793, 1935.
(13) EBERT, R. V., E. A. STEAD ET AL. Am. J. Med. Sci. **201:** 655, 1941.
(14) HAHN, P. F., W. F. BALE AND W. M. BALFOUR. This Journal **135:** 600, 1942.
(15) ELMAN, R. J. A. M. A. **120:** 1176, 1942.

THE RELATIONSHIP OF THE DIABETOGENIC EFFECT OF DIETHYLSTILBESTROL TO THE ADRENAL CORTEX IN THE RAT

DWIGHT J. INGLE

From the Research Laboratories, The Upjohn Company, Kalamazoo, Michigan and The George S. Cox Medical Research Institute, The University of Pennsylvania, Philadelphia

Received for publication October 9, 1942

Diethylstilbestrol has been shown (1) to be diabetogenic in partially depancreatized and in normal force-fed rats. Since certain of the adrenal cortical hormones are also diabetogenic (2) and since the adrenal cortices hypertrophy during the administration of diethylstilbestrol (3) it is reasonable to consider the hypothesis that the diabetogenic effect of this substance is due to its stimulation of the adrenal cortex to secrete sufficient amounts of its hormones to cause glycosuria. In this study it is found that diethylstilbestrol exerts some diabetogenic effect which is not mediated through the adrenal cortex although the presence of the adrenal cortical hormones is required for the full manifestation of severe glycosuria.

METHODS. Male rats of the Sprague-Dawley strain were used. Partial pancreatectomy was carried out at a body-weight of 250 to 300 grams. All of the pancreas was removed except that portion between the bile duct and the duodenum. The technique used in earlier studies (4) was modified according to Richter (5) so that the pancreas lying within the duodenal loop was removed by suction through a small pipette. By this modification it is possible to remove up to 95 per cent of the pancreas in a single stage operation in rats of any body-weight with less than 20 per cent mortality. The technique used for adrenalectomy has been described (4).

Following partial pancreatectomy the rats were maintained on a diet of Purina dog chow until they had reached a weight of 325 to 375 grams. They were then placed in metabolism cages and maintained on a fluid diet administered by stomach tube each morning and late afternoon. The technique of force-feeding and the diets used were modifications of the methods described by Reinecke, Ball and Samuels (6). The diets used were made up according to the table (see p. 578).

During the period of adaptation to force-feeding the diets were first administered in small amounts to prevent the development of "food shock" (1). The animals were brought to a full feeding on the 8th day. Each rat received 26 cc. of diet per day. The total available carbohydrate of diet A was approximately 6.5 grams; and the total available carbohydrate of diet B was approximately 5.0 grams. These values are based on analyses of the diet and assume that the maximum formation of glucose from protein does not exceed a glucose:nitrogen ratio of 3.6.

The 11-desoxycorticosterone acetate was dissolved in sesame oil (5.0 mgm.

CONSTITUENT	DIET A	DIET B
Egg albumin (Merck)	200 grams	
Cellu flour (Chicago Dietetic Supply)	120 grams	120 grams
Osborne & Mendel salt mixture	40 grams	40 grams
Vitamin preparation (Vi-Penta. Roche)	10 cc.	
Wheat germ oil	10 cc.	10 cc.
Mazola oil	10 cc.	175 cc.
Cod liver oil	10 cc.	10 cc.
Starch	200 grams	
Dextrin	100 grams	
Sucrose	100 grams	
Butter fat	295 grams	
Dried yeast (Fleischmann)		100 grams
Whole milk powder (Merrell-Soule)		600 grams
Water to make total volume of	2000 cc.	2000 cc.

per cc.) and administered in divided doses (2.0 mgm. per day) each morning and late afternoon. The diethylstilbestrol was dissolved in sesame oil (1.0 mgm. per cc.) and doses of 0.1 mgm. were administered once daily. The adrenal cortical extract was made up in 0.9 per cent sodium chloride solution and represented 40 grams of beef adrenal glands per cubic centimeter. It was administered in divided doses (3 cc. per day) each morning and late afternoon. All solutions were administered subcutaneously. Twenty-four hour specimens of urine were collected at the same hour each day and preserved with thymol. The determination of urinary glucose was carried out according to the method of Benedict (7) and that of blood glucose by the method of Miller and Van Slyke (8).

Experiments and results. Experiment 1 was a study of the effect of diethylstilbestrol in the adrenalectomized, depancreatized rat maintained on 11-desoxycorticosterone acetate. Six rats were partially depancreatized. All of the pancreas was removed except for approximately 50 per cent of the tissue which lies between the bile duct and the duodenum. During the experiment they were maintained on diet A. Prior to adrenalectomy each of the rats spontaneously excreted glucose in amounts up to 1.8 grams daily. Following removal of the adrenal glands each animal was maintained on 2.0 mgm. daily of 11-desoxycorticosterone acetate during the remainder of the experiment and under these conditions each animal was free from glycosuria until diethylstilbestrol was administered. Following a control period of 10 days each animal was treated with 0.1 mgm. diethylstilbestrol daily until it died within a period of 5 to 11 days. During the period of treatment with diethylstilbestrol each animal developed a mild glycosuria and then, within one or two days, developed symptoms of diabetic shock and died. Determinations of blood sugar showed that symptoms of shock occurred at a time when the level of blood glucose was above 200 mgm. per cent. The data on urinary glucose are summarized in figure 1.

Experiment 2 was a comparison of the diabetogenic effect of diethylstilbestrol

in the partially depancreatized rat prior to adrenalectomy and following adrenalectomy when different types of replacement therapy were used to control adrenal cortical insufficiency. Five rats were partially depancreatized by removing all of the pancreas except the portion between the bile duct and the duodenum which was left intact. During the experiment they were maintained on diet B. Each rat was free of spontaneous glycosuria and all of them responded to the administration of diethylstilbestrol by developing a severe glycosuria prior to adrenalectomy and also during two test periods following adrenalectomy when the animals were treated with adrenal cortical extract. However, when the treatment with adrenal cortical extract was replaced by the administration of 11-desoxycorticosterone acetate only one of the five rats developed glycosuria during the administration of diethylstilbestrol. Similarily,

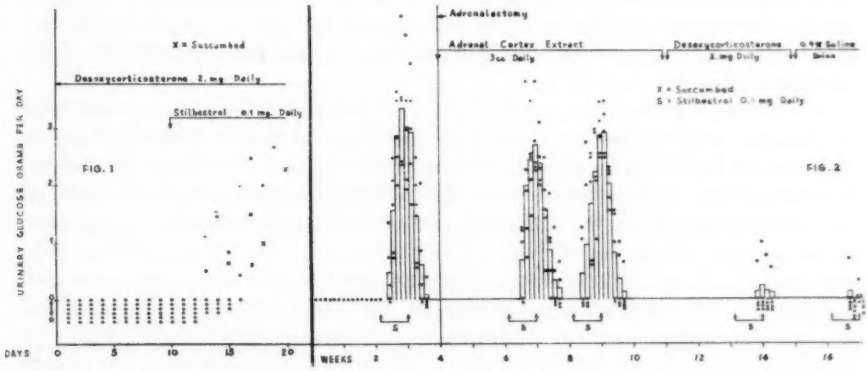

Fig. 1 Fig. 2

Fig. 1. A diabetogenic effect of diethylstilbestrol in the adrenalectomized, partially depancreatized rat treated with 11-desoxycorticosterone acetate.

Fig. 2. The diabetogenic effect of diethylstilbestrol before and after adrenalectomy of the partially depancreatized rat as influenced by the nature of the replacement therapy.

when the animals were mantained by drinking 0.9 per cent solution of sodium chloride, two rats excreted a small amount of glucose for only one day. All of the rats died as a result of the administration of diethylstilbestrol during the period of maintenance on solution of sodium chloride. Prior to this phase of the experiment all of the rats gave the appearance of being in good health and vigor at all times. The data on urinary glucose and the periods of treatment are summarized in figure 2.

Discussion. It is clear from these experiments that diethylstilbestrol exerts some effect upon carbohydrate metabolism of the rat which is not mediated by the adrenal cortex. The presence of the adrenal cortical hormones such as occur in adrenal cortical extract is necessary for a full manifestation of the diabetogenic activity of diethylstilbestrol since glycosuria is either reduced or absent when these same test animals are maintained on 11-desoxycorticosterone acetate or by drinking 0.9 per cent solution of sodium chloride.

The amounts of adrenal cortex extract which were administered in these experiments were large as judged by the small amount of this extract which is required to maintain life in adrenalectomized rats (3 rat units per cc.) but it is well established that large doses are required to maintain normal carbohydrate metabolism in adrenalectomized rats and massive doses are required before diabetogenic effects of adrenal cortical extracts can be demonstrated (9). These experiments do not prove that none of the effect of diethylstilbestrol on the carbohydrate metabolism of the non-adrenalectomized rat is due to an increased activity of the adrenal cortex. The intensity of the glycosuria induced by diethylstilbestrol following adrenalectomy and during the period of adrenal cortex administration was less than that manifested prior to adrenalectomy. This difference may have been due to chance; it may have been due to a failure of the replacement therapy to provide as much cortical hormone as is normally secreted by the cortex; or it is conceivable that a part, but not all, of the diabetogenic effect of diethylstilbestrol is brought about by an increased activity of the adrenal cortex.

The adrenal cortex undergoes hypertrophy in animals subjected to any type of stress or damaging agent. Although it is probable that its secretory activity is increased during these periods of enlargement, proof is lacking. Similarily, it has not been proven that the enlarged adrenal cortex causes any abnormal physiologic changes in the organism similar to those induced by over-dosage of the adrenal extracts or cortical steroids. Ingle (1) studied the effect of subjecting diabetic rats to stress and damaging agents of the sort which produced marked adrenal cortical hypertrophy on the assumption that an increased secretion of adrenal cortical hormones might intensify the diabetic state just as will the injection of these hormones. The results were negative. It is conceivable that the increased secretion of hormones by the hypertrophied cortex would not intensify the diabetic state or produce other abnormal changes because under conditions of stress the increased secretion of hormones would only suffice to meet a physiologic need. Thus homeostasis would be maintained rather than being disturbed. If this hypothesis is true, the adrenal cortical hypertrophy which follows the administration of diethylstilbestrol may be necessary for a full manifestation of the diabetogenic effect of diethylstilbestrol, not because adrenal cortical hormones are being secreted in diabetogenic amounts, but because increased amounts of them are required to maintain the functional normality of those mechanisms essential for the metabolism of carbohydrate.

The failure of diethylstilbestrol to manifest its full diabetogenic effect in the absence of the adrenal cortical hormones may be due in part to the lowering of the carbohydrate stores of the body and to the high rate of glucose oxidation during adrenal insufficiency (10). Larger amounts of glucose would be required to exceed the kidney threshold for glucose in the case of the carbohydrate depleted adrenalectomized rat than in the case of the non-adrenalectomized rat. The relative importance of this and other undefined factors in causing the diminished response to diethylstilbestrol in the absence of the cortical hormones

cannot now be evaluated. It should be pointed out that the effect of pancreatectomy and of anterior pituitary extracts on carbohydrate metabolism is diminished in adrenalectomized animals but in each case can be restored by treatment with adrenal cortical extract (2).

It is significant that in experiment 1, the adrenalectomized, partially depancreatized rats treated with 11-desoxycorticosterone acetate developed shock at a time when hyperglycemia was present. Partially depancreatized rats having their adrenals intact and partially depancreatized, adrenalectomized rats treated with large amounts of adrenal cortical extract are able to resist a severe diabetic state without developing symptoms of shock. The lower than normal resistance of the animals studied in experiment 1 was therefore due to lack of some cortical hormone. Under the usual experimental conditions shock states in animals having cortical insufficiency are characterized by hypoglycemia and it is frequently assumed that depletion of the carbohydrate stores of the body is a factor in causing the low resistance of adrenalectomized animals to shock. In these experiments the animals were treated with desoxycorticosterone acetate which is believed to prevent deficiencies in electrolyte metabolism and the additional treatment with diethylstilbestrol raised the blood glucose to abnormally high levels without preventing shock. This may be taken as evidence that the effect of the adrenal cortex on resistance can be partially dissociated from deficiencies in carbohydrate and electrolyte metabolism.

SUMMARY

Six partially depancreatized rats which had a mild spontaneous glycosuria were adrenalectomized and treated with 2.0 mgm. of 11-desoxycorticosterone acetate daily. Following adrenalectomy the glycosuria disappeared but was reinduced by the administration of 0.1 mgm. of diethylstilbestrol daily. After a few days each rat died although the glycosuria was not severe. Symptoms attributable to adrenal cortical insufficiency were found to coexist with a state of hyperglycemia.

In a second experiment, five partially depancreatized rats which were without spontaneous glycosuria became diabetic during the administration of 0.1 mgm. of diethylstilbestrol daily and the glycosuria disappeared when the diethylstilbestrol was withdrawn. These animals were adrenalectomized and maintained on 3.0 cc. daily of adrenal cortical extract. Glycosuria developed only when diethylstilbestrol was administered. When these animals were maintained by treatment with 11-desoxycorticosterone acetate or by drinking a solution of 0.9 per cent sodium chloride the diabetogenic effect of diethylstilbestrol was either slight or absent.

Acknowledgment. The author wishes to express his appreciation to Dr. G. A. Harrop, The Squibb Institute for Medical Research, New Brunswick, New Jersey, for supplying the diethylstilbestrol; to Dr. E. Schwenk, The Schering Corporation, Bloomfield, New Jersey, for supplying the 11-desoxycorticosterone

acetate, and to Dr. M. H. Kuizenga of this laboratory for supplying the adrenal cortical extract.

REFERENCES

(1) INGLE, D. J. Endocrinology **29:** 838, 1941.
(2) LONG, C. N. H., B. KATZIN AND E. G. FRY. Endocrinology **26:** 309, 1940.
(3) SELYE, H. Canadian M. A. J. **41:** 48, 1939.
(4) INGLE, D. J. AND J. Q. GRIFFITH. Chapter 16. The rat in laboratory investigation. J. B. Lippincott Co., Philadelphia, 1942.
(5) RICHTER, C. P. AND E. C. H. SCHMIDT, JR. Endocrinology **25:** 698, 1939.
(6) REINECKE, R. M., H. A. BALL AND L. T. SAMUELS. Proc. Soc. Exper. Biol. and Med. **41:** 44, 1939.
(7) BENEDICT, S. R. J. A. M. A. **57:** 1193, 1911.
(8) MILLER, B. F. AND D. D. VAN SLYKE. J. Biol. Chem. **114:** 583, 1936.
(9) LONG, C. N. H. Transactions and studies of the College of Physicians of Philadelphia **7:** 21, 1939.
(10) THORN, G. W., G. F. KOEPF, R. A. LEWIS AND E. F. OLSON. J. Clin. Investigation **19:** 813, 1940.

THE INFLUENCE OF INTERELECTRODAL DISTANCE IN ELECTRICAL STIMULATION OF NERVE AND OF STRIATED AND VENTRICULAR MUSCLE

A. ROSENBLUETH AND G. H. ACHESON

From the Department of Physiology in the Harvard Medical School

Received for publication October 12, 1942

This study was prompted by the observation that the distance between two stimulating electrodes applied to the turtle's ventricle did not modify the threshold. As is well known, the threshold of motor nerve fibers increases markedly when the interelectrodal distance is shorter than about 5 to 3 mm. It was considered interesting to compare in this respect A and C nerve fibers, and also striated and ventricular muscle.

METHOD. The nerves studied were the A and C fibers in the saphenous of cats under dial anesthesia (Ciba, 0.7 cc. per kgm., intraperitoneally). The nerves were dissected, as free from fascia as possible, from the origin at the femoral nerve to below the knee; a few branches were cut in the process. The excised nerves were laid on parallel fine wires (diameter 0.2 mm.) placed at various fixed distances in a moist chamber. The stimuli were usually applied to the central part of the nerve, while the electric responses were recorded from the peripheral end; the reverse procedure, occasionally tested, yielded similar results.

With the cathode in a given position the interelectrodal distance was first increased and then decreased by using different anodes. Since the electrodes in the chamber provided only fixed distances, in order to test as many intervals as possible it was necessary to shift the cathode to different points. There were usually no significant discrepancies with the several cathodes.

The influence on the thresholds of the changes of resistance caused by varying interelectrodal distance was minimized whenever possible by putting a resistance of 10,000 to 250,000 ω in series with the nerve.

The fine wires used as electrodes were quite polarizable. A cumulative effect was prevented by placing a resistance (5,000 or 20,000 ω) in parallel with the nerve and the series resistance. Polarization led to some scatter of the measurements but not so great as to mask the phenomenon studied.

The stimuli were condenser discharges of various capacities (0.001 to 1 μF). The discharging circuit was such that the time constant of the discharges was not significantly affected by the change of position of the electrodes on the nerve. Qualitatively similar results were obtained with all the capacities tested.

The electric responses were led monophasically to a cathode-ray oscillograph after suitable amplification. They were measured visually on the oscillograph. By threshold is meant the voltage necessary to elicit a constant submaximal response (about 30 per cent of maximal).

The striated muscles studied were the sartorii of the cat and frog. They were exposed by opening the skin of the pithed frogs or the cats. Desiccation was prevented by covering with mineral oil or, preferably, by intermittent application of the appropriate Ringer's solution which was removed before the observations.

The stimulating electrodes were steel needles with a tip of about 0.1 mm. One, the cathode, was in a fixed position. The other, the anode, was supported by a mechanical stage of a microscope and could be placed with ease at any desired position. Both electrodes were applied to the surface of the muscles. A dissecting microscope with a micrometer permitted the measurement of the interelectrodal distance with an accuracy better than 0.1 mm.

The electric responses of the muscles were led to the cathode-ray oscillograph. As shown by Rosenblueth (1940), the latency of the electrograms permits the separation of responses to direct stimulation from those due to indirect activation. The direct responses were measured as above.

The ventricles, separated from the atria in pithed turtles, were pinned to a board and kept moist. The placement of the stimulating electrodes was as for striated muscle. The response observed was the ventricular contraction.

RESULTS. The observations on A fibers confirm previous reports (see for references Rushton, 1927, 1934). The upper curve in figure 1 illustrates a typical experiment. The results are plotted in logarithmic scales. For the abscissae, this type of scale avoids the crowding of small interelectrodal distances; and for the ordinates, it allows a ready estimation of the ratio of the threshold at a given short distance to that at long distances. The thresholds are in conventional units.

When the changes of nerve resistance with variable interelectrodal distance could influence the distribution of current—i.e., when no resistance was placed in series with the nerve—there was a distance at which the threshold was minimal, and shorter or longer distances required higher voltage for stimulation. This was true in the experiment illustrated in figure 1; with longer interelectrodal distances than those included in the graph the threshold rose. The absence of this minimum in the observations with a high series resistance confirms Rushton's (1927, 1934) results.

The observations on C fibers in the cat's saphenous nerve were qualitatively similar to those on A fibers. Significant quantitative differences were that the threshold rise occurred only at shorter distances and that it was not as striking for the C as for the A fibers (cf. the upper and the middle curves in fig. 1). For reasons which remain obscure, the results on C fibers were more irregular than those on A fibers. The A curve was fairly constant from nerve to nerve with the same experimental conditions, while the C curve could vary markedly. Invariably, however, the effect of short interelectrodal distance was more striking for the A than for the C elements of the same nerve.

In contrast with the results obtained in nerve fibers, the interelectrodal distance had no influence on the threshold of striated and ventricular muscle (lower curve of fig. 1). Even with shorter intervals than those represented, e.g., 0.15 mm., the threshold was not different from that with a distance of 1 cm.

The problem of the influence on threshold of the angle made by the electrodes and the excitable elements is closely related to the topic of this study (see Rushton, 1927). In the sartorius muscles several observations were made in which the interelectrodal distance was constant (about 1 mm.) while the angle was varied from 0 to 90° by changing the position of the anode. No significant influence of this angle on threshold was detected.

DISCUSSION. According to Rushton (1934) the influence of interelectrodal distance on the threshold of A fibers is due to the structure of nerves. Nerve fibers are assumed to consist of conducting cores and resistant sheaths. With

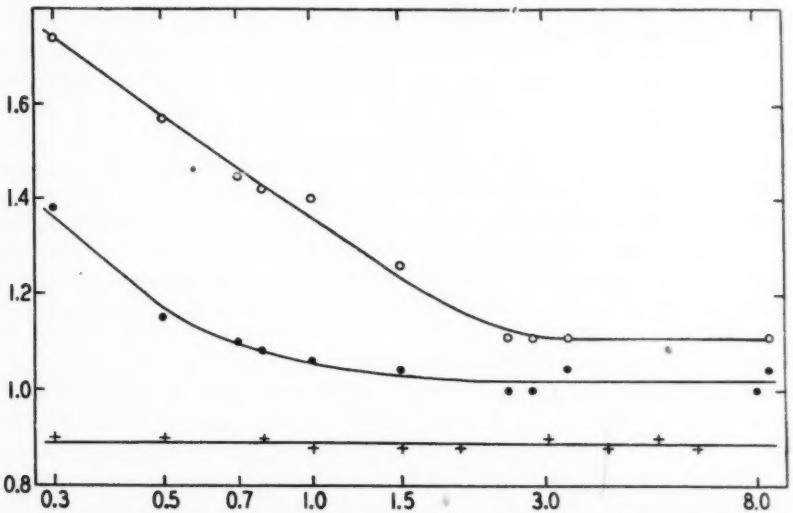

Fig. 1. Relations of threshold to distance between stimulating electrodes. Abscissae: distance in millimeters. Ordinates: threshold voltage in conventional units. The scales are logarithmic.

Upper curve (circles): nerve A fibers. Capacity of the stimulating condenser: 0.5 μF. Resistance in parallel with the nerve: 5,000 ω.

Middle curve (dots): nerve C fibers. Stimulating circuit as for the A fibers.

Lower curve (crosses): ventricular muscle. Capacity: 0.1 μF. Resistance in series with muscle: 20,000 ω. Resistance in parallel: 20,000 ω.

a long interelectrodal distance the current through the cores would be a relatively large fraction of the total current flow. With short distances, on the other hand, only a small fraction of the total current would flow through the fibers, most of it passing through the surrounding connective tissue and interstitial fluid.

The application of this theory to the present data implies that A fibers are relatively well insulated, possibly by the myelin sheath. C fibers have less insulation. Finally, striated and ventricular muscles behave as if they were practically uninsulated.

SUMMARY

With short distances between the stimulating electrodes, the threshold of C nerve fibers rises significantly, but not as much as does that of A fibers (fig. 1).

The threshold of striated and ventricular muscle to electrical stimuli is independent of the interelectrodal distance (fig. 1). The threshold of striated muscle is also independent of the angle between the stimulating current and the muscle fibers.

REFERENCES

ROSENBLUETH, A. This Journal **129:** 22, 1940.
RUSHTON, W. A. H. J. Physiol. **63:** 357, 1927.
Ibid. **82:** 332, 1934.

EXPERIMENTALLY INDUCED HYPERTENSION IN PARABIOTIC RATS[1]

ARTHUR GROLLMAN AND COLTER RULE

From the Department of Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.

Received for publication October 12, 1942

Despite the mass of experimental data which has accumulated in recent years on the subject of hypertension, conclusions regarding the exact rôle of the kidney in this process are still a matter of dispute. The available facts have been interpreted in several ways. Some assume the liberation of a pressor substance (angiotonin, hypertensin) in the renal vein in sufficient concentration to be detectable by perfusion through an isolated rabbit's ear, or toad, or by injection into animals. Others, although accepting the existence of a renal pressor substance, do not consider that renin or its derivatives (angiotonin, hypertensin) are the agents responsible for hypertension. According to many, the relative balance between "anti-pressor" and "pressor" substances, both of which are elaborated by the kidney, determines the existence of hypertension. And finally, the hypothesis has been advanced that hypertension results simply from a deficiency of a renal factor without the implication of any pressor substance.

It is now generally admitted, however, that experimental renal hypertension is mediated by a humoral mechanism. If this be the case, the reaction of parabiotically united animals should throw light on the mechanism involved in hypertension, as it has in the case of the various hormonal reactions in which this procedure has been utilized. The blood pressures of such parabiotic rats have been determined following various operative procedures on the kidneys and the results interpreted in the light of current theories regarding the etiology of experimental hypertension.

METHODS. A total of over 50 pairs of rats have been joined in parabiosis by the technique described by Bunster and Meyer. In this procedure skin and muscles throughout the length of the animals are united, but the abdominal cavities remain separated. Animals 20 to 30 days of age, weighing 15 to 40 grams, were used. They were of a pure Wistar strain and were litter mates matched for size and sex. Successfully united pairs were allowed to attain maturity and their blood pressures determined daily throughout subsequent procedures. The blood pressure was determined on the unanesthetized animals by the plethysmographic method of Williams, Grollman and Harrison. A special holder was devised which permitted measurements to be made on each rat of a pair alternately until constant results were obtained, as in determinations on single animals.

Hypertension was induced by applying cotton cloth to the kidney. In some

[1] Aided by a grant from the John and Mary R. Markle Foundation.

cases this kidney was subsequently removed; in others the remaining normal kidney of the same rat or one of the kidneys of its co-twin were removed. In all instances, however, at least a month elapsed, during which pressures were taken daily, before proceeding to the next operation.

RESULTS. *The blood pressure in parabiotic rats.* The systolic blood pressure in the individual members of a parabiotic pair were not always identical. However, the pressure in each animal remained within a normal range and the variations between members of a pair did not exceed the daily variations observed in each individual rat. A typical series of readings taken during the course of a week on 2 pairs of parabionts is reproduced in table 1. In view of the magnitude of the variations in each individual rat from day to day, it is impossible to conclude that the variations between the members of a pair are real and not due to uncontrollable errors in the readings. However, since, as shall be shown later, the blood pressures of members of a pair may vary widely, it is probable that the

TABLE 1

The individual daily blood pressure readings on two pairs of parabiotic rats during the course of a week

Values are expressed as millimeters of mercury

Pair I:							
Right member	120	120	130	140	130	120	120
Left member	110	120	130	130	140	120	130
Pair II:							
Right member	125	130	120	125	120	120	120
Left member	110	120	130	130	120	110	120

observed differences are real and illustrate the independence of the factors regulating the blood pressure level in each rat.

The application of cloth to one kidney. As is the case in separate animals (Grollman and Williams) the application of cloth to a single kidney of one of a pair of parabiotic rats was variable in its effects. In many pairs, such an operation induced no demonstrable changes in the blood pressure of either member of the pair. In the majority of experiments, the rise in blood pressure of the rat in which cloth had been applied to one kidney was only 10 to 20 mm. of mercury, while that of the intact co-twin was within the limits of daily normal variation. In these animals the blood pressure gradually attained a maximum level 10 to 20 mm. above its preoperative level about 10 days following the operation, gradually resuming its normal level after the elapse of another 10-day period.

The third type of reaction following the application of cloth to one kidney of a single member of a parabiotic pair is reproduced in figure 1. In this group of animals the blood pressure of the rat with cloth on one kidney rose gradually to hypertensive levels and remained elevated throughout the subsequent period of observation. The blood pressure of the co-twin, on the other hand, either

remained at its preoperative level as in figure 1, or was only slightly elevated. The response shown in figure 1 illustrates this marked dissociation which may occur between the blood pressure levels of parabiotically united animals.

The application of cloth to one kidney with ablation of the other. In parabionts remaining normotensive after the application of cloth to one kidney, the removal of the remaining normal kidney from the same rat invariably resulted in an elevation of blood pressure in the operated animal to hypertensive levels (150 to 180 mm.). The rise in pressure occurred gradually during the period of a week or ten days following the nephrectomy and rose thereafter at a slow rate until the death of the animal (fig. 2). This is essentially the reaction noted in single rats following similar operations (Grollman and Williams).

The systolic blood pressure of the intact co-twin following the above-described operations was variable. In many instances it remained within normal limits

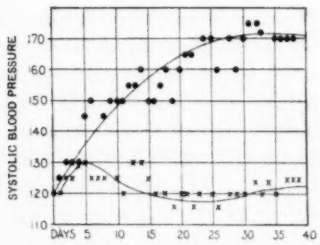

Fig. 1

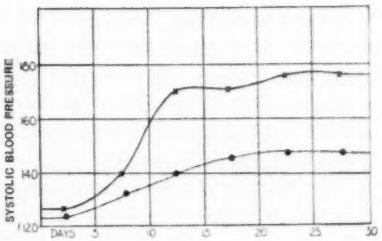

Fig. 2

Fig. 1. The effect of applying a cloth capsule to a single kidney of one of a pair of parabiotically united rats. The upper curve (—●—) shows the blood pressure response of the rat which was operated upon; the lower curve (—x—), of the co-twin, the kidneys of which remained intact. Systolic blood pressures are expressed in millimeters of mercury.

Fig. 2. The effect of applying a cloth capsule to one kidney and removing the other from one of a pair of parabiotically united rats. The upper curve (—x—) shows the blood pressure response of the rat which was operated upon; the lower curve (—●—) of the co-twin, the kidneys of which remained intact. Each point is the average of daily determinations over a period of five days. Systolic blood pressures are expressed in millimeters of mercury.

of variation, so that the blood pressure curve of the two animals was identical to that of figure 1. In most cases, however, the blood pressure of the intact animal rose moderately as illustrated in figure 2. There remained a complete dissociation of the blood pressure levels in the two animals, as in the case illustrated in figure 1.

In only one instance did the systolic blood pressures of both rats approximate the same level. In this case the average systolic pressure of the operated member of the pair was 150 mm., while that of the unoperated one rose to 140 mm.

Bilateral nephrectomy. Extirpation, which affords so valuable a method for demonstrating the hormonal function of the glands of internal secretion, cannot be applied to the kidney because of the short survival period of animals following nephrectomy. Both kidneys of one of a pair of parabiotic rats may, however, be removed and the animals remain in relatively good condition for long periods

(Hermannsdorfer). Jeffers and his collaborators have recently utilized this method and demonstrated that following the removal of three of the available 4 kidneys, hypertension results in the nephrectomized rat. We have confirmed this finding on two pairs of parabionts. In our experiments, however, the removal of the kidneys from only one rat sufficed to induce hypertension, the blood pressure of the nephrectomized animal rising to 170 to 190 mm., while that of the co-twin rose only to 130 to 140 mm. The reaction was thus similar to that shown in figure 2.

Discussion. The results of the present study demonstrate that by the procedure of parabiosis used, it is possible to maintain animals in union and nevertheless have a striking dissociation of the blood pressure of the individuals. Parabiotic union as performed results in a fine network of anastomosing capillaries penetrating between the parabionts. Although no large vessels pass between them, the exchange suffices to permit relatively rapid interchange of constituents between the blood of the two partners. This can be demonstrated by the injection of a dye into one animal and noting its rate of accumulation in the co-twin. Thus, following the injection of $\frac{1}{2}$ cc. of a $\frac{1}{2}$ per cent solution of Evans Blue into the tail vein of one rat, the relative amounts of dye in the blood were 54 and 46 per cent in the injected and uninjected twins, respectively, after two hours. Our results confirm those reported by Hill, using brilliant vital red, and need, therefore, not be repeated here. In the case of a rapidly excreted dye (phenolsulfonephthalein), a total of 80 per cent of the dye was excreted in three hours, 70 per cent by the injected rat and 10 per cent by its co-twin. In the case of a more rapidly destroyed substance (epinephrine) it was found that whereas this substance induced the usual pressor effect in the injected rat, no rise in blood pressure could be detected in the co-twin.

The variability in the distribution of a very rapidly utilized (epinephrine), a relatively rapidly excreted (phenolsulphonphthalein) and a slowly excreted substance (Evans Blue) between the members of a parabiotic pair exemplifies the type of reactions which one encounters in the distribution of normally excreted endocrine substances. In the case of the female sex hormone, for example, the secretion of one animal exerts only a slight effect on the estrous cycle of its partner; a normal female only rarely induces estrus in its ovariectomized co-twin; and union of a male and a female does not inhibit estrus in the latter (Hill). This demonstrates that the threshold of hormone necessary for maintaining the estrous cycle is seldom reached in the blood of the castrated parabiont. On the other hand, the hormones of pregnancy affect the non-pregnant co-twin promptly (Hill). A similar difference in the ease with which homogeneity of hormonal environment is attained in parabionts has been demonstrated by Witschi for the melanophore and metamorphosing hormones. The former is much slower in attaining its effective concentration in hypophysectomized parabiont than is the latter.

The physiological effectiveness of a transmitted humoral agent between two animals in parabiosis depends apparently on the abundance with which it is produced and its threshold concentration in the blood necessary for inducing its physiological activity (Witschi, Hill). A humoral agent produced constantly

and present in the blood in appreciable concentration, compared to its concentration in the tissues, will ultimately distribute itself between and affect both parabionts. On the other hand, if released only in small amounts and removed rapidly by the tissues, only a minimal concentration will remain in the blood and the co-twin will manifest a deficiency of the hormone in question. Assuming that the kidney elaborates a humoral substance involved in the causation of hypertension, the present experiments indicate that the kidney normally does not release an overdose of this substance but merely produces an adequate amount for the needs of the organism. The kidney apparently cannot greatly increase its output of the hypothetical substance with an increase in demand incurred by the experimental procedures employed in inducing hypertension.

At first sight, the results of the present study would seem to exclude the possibility of explaining the occurrence of hypertension by any humoral agency. However, the results obtained in the case of generally accepted endocrine reactions (*e.g.*, the phenomenon of estrus, cited above) show that it is possible to have a dissociation of effects on the members of a pair of parabionts even in the case of humoral substances, if the latter be present in the blood in amounts much less than the threshold level necessary for inducing its specific action. The present experiments are compatible with the views that the kidney elaborates 1, pressor, or 2, anti-pressor substances, or 3, a humoral agent in the absence of which hypertension results.[2] The results, however, do exclude the possibility that any humoral agent involved is present in the blood stream in sufficient concentration to induce its effects directly.

The present findings are consistent with the view that the kidney elaborates a substance which is essential for the organism and in the absence of which hypertension results, or that such a substance exerts an "anti-pressor" activity to antagonize the action of some pressor substance. The fact that bilateral nephrectomy alone of one of a pair of parabionts results in an elevation of blood pressure, would favor the first and simpler view, for it is difficult to see how simple ablation of renal tissue could induce an imbalance between the amounts of "pressor" and "anti-pressor" substances.

SUMMARY

Rats were joined in parabiotic union and their systolic blood pressures determined daily following operative procedures on the kidney. Parabiotic individuals retain an independence of their circulatory adjustments; hypertensive blood pressure levels may be maintained in one member of a parabiotic pair, while the blood pressure of the co-twin remains normal. In some instances, however, the hypertensive action induced by procedures on the kidney is trans-

[2] The view that hypertension results from the absence of some essential constituent from the organism may appear incongruous on superficial consideration. However, it is analogous to the rise in blood pressure which occurs in anoxemia, where it is the absence of oxygen which induces changes which in turn cause the observed change in blood pressure. Similarly, the lack of some incretory product of the kidney (Grollman) may result in changes in the organism which in turn give rise to hypertension.

mitted to the intact co-twin. The results are interpreted as being most consistent with the view that the kidney normally elaborates a substance necessary for the maintenance of normal blood pressure levels. The bearing of the results on other current theories of the pathogenesis of experimental renal hypertension is discussed.

REFERENCES

BUNSTER, E. AND R. K. MEYER. Anat. Rec. **57:** 339, 1933.

GROLLMAN, A. Essentials of endocrinology. J. B. Lippincott Co., Philadelphia, 1941.

GROLLMAN, A. AND J. R. WILLIAMS, JR. Am. J. Med. Sci. **204:** 73, 1942.

HERRMANSDORFER, A. Deutsch. Ztschr. f. Chir. **178:** 289, 1923.

HILL, R. T. J. Exper. Zool. **63:** 203, 1932.

JEFFERS, W. A., M. A. LINDAUER, P. H. TWADDLE AND C. C. WOLFERTH. Am. J. Med. Sci. **199:** 815, 1940.

WILLIAMS, J. R., JR., T. R. HARRISON AND A. GROLLMAN. J. Clin. Investigation **18:** 373, 1939.

WITSCHI, E. Proc. Soc. Exper. Biol. and Med. **28:** 869, 1931.

VENOUS PRESSURE AND CIRCULATION TIME DURING ACUTE PROGRESSIVE ANOXIA IN MAN[1]

I. ERSHLER, C. E. KOSSMANN AND M. S. WHITE

From The Department of Aviation Medicine, and the Laboratory for Clinical Investigation, The School of Aviation Medicine, Randolph Field, Texas

Received for publication October 12, 1942

Information concerning the venous pressure and the circulation time during anoxia in man is limited and inconclusive. Schneider and Truesdell (1), using the indirect method of Hooker and Eyster (2), found a decrease in the venous pressure during the acute anoxia induced by rebreathing, or by dilution of the respired air with nitrogen, or by reduction of the atmospheric pressure in a chamber. The decrease was attributed to splanchnic vasodilatation. Levy, Barach and Bruenn (3) observed the effects of breathing a mixture of 12 per cent oxygen and 88 per cent nitrogen for a period of approximately twenty minutes. In eighteen patients with heart disease, the arm to tongue circulation time, with two exceptions, was reduced. In eleven normal subjects relatively small changes in the circulation time were noted, although ten showed a decrease. There was no definite trend of the variations in the direct venous pressure in the abnormal subjects; it was not measured in the normals.

METHODS. Nineteen normal volunteers from the enlisted personnel stationed at Randolph Field, Texas, were subjected to progressive anoxia induced by rebreathing. In the rebreather[2] the utilized oxygen was automatically replaced by nitrogen and the carbon-dioxide was absorbed by soda-lime.

The venous pressure was measured continuously by the Moritz von Tabora direct method (4) in an antecubital vein of the right arm. An 18 gauge needle was used in the first few experiments; in the remainder a 15 gauge needle was used. The skin over the selected vein was anesthetized with a 2 per cent solution of novocain before introducing the needle. The arm was placed on the bed at the same level as the subject's body. The zero level of the manometer was set 5 cm. below the fourth right costo-chondral junction. A 5 per cent solution of sodium citrate was the anticoagulant. The entire system was kept free of clots by frequent small irrigations with citrate solution stored in a reservoir connected with the apparatus.

The circulation time from the right arm to the tongue was determined by the decholin method (5). The amount of decholin used varied from 3 cc. to 5 cc. of a 20 per cent solution. It was injected rapidly and the time was measured by a stop watch from the end of the injection until the subject first perceived a bitter taste.

Arterial pressure was determined by the auscultatory method with a mercury

[1] Presented at the Thirteenth Annual Meeting of the Aero Medical Association of the United States, at Boston, Massachusetts, November 1, 1941.

[2] Designed and constructed by Lt. Col. N. W. White, M.C., U. S. Army.

manometer. Heart rates were counted with a stop watch and frequently recorded by the electrocardiograph. During the final five minutes of each rebreathing period and for the first few minutes after the subject again breathed room air, the electrocardiogram was recorded continuously.

Oxygen saturation of the ear blood was determined continuously throughout the course of each experiment by the photocell oximeter of Millikan (6). Checked by chemical analyses this method has been shown to have an accuracy within 5 per cent down to arterial oxygen saturations of 75 per cent, and within 8 per cent below that level. With this method the normal oxygen saturation is assumed to be 96 per cent.

Respiratory volumes were determined from the spirograms. These were corrected for dry air at 0 degrees C. and 760 mm. of mercury pressure.

Each experiment was conducted in an air conditioned room at a temperature of approximately 27°C. (80°F.) as follows: The subject reclined for at least thirty minutes before the rebreathing was started. During this time all variables except the pulmonary ventilation were measured. During the rebreathing period, which varied from twenty to twenty-five minutes in length, the oxygen saturation of the ear blood, the venous pressure, and the pulmonary ventilation were recorded continuously. The pulse rate and the arterial blood pressure were determined at frequent intervals. The circulation time was measured when the oxygen saturation was approximately 85 per cent and again when it was 75 per cent. At the end of the rebreathing period the subject breathed room air. All variables except the pulmonary ventilation were again measured for at least five minutes or until they had reached basal levels.

The volume of the packed red blood cells was determined in seventeen subjects at various levels of anoxia down to 75 per cent oxygen saturation of the blood. In fourteen of these individuals the changes were slight; in three they were marked. Since the data seem insufficient for any conclusion, further reference to them will be omitted at this time.

Results. Figure 1 shows one type of response to the anoxia induced by rebreathing. In subject 18 (fig. 1) there was a sudden drop in the systolic, diastolic, and pulse pressures and an abrupt rise in the venous pressure when the oxygen saturation of the ear blood was 64 per cent. At the time the subject complained of feeling faint. In this series seven subjects (37 per cent) showed a similar "circulatory crisis."

Subject 20 displayed a rise in the systolic and pulse pressures, and a gradual fall in the venous pressure (table 1) as the oxygen saturation of the ear blood approached 65 per cent. The rebreathing period was terminated because the subject showed some clouding of consciousness. This "non-fainting" type of reaction to anoxia was observed in twelve subjects (63 per cent).

Venous pressure. The results of eighteen experiments are shown in table 1. In each subject the initial or control venous pressure is the average of at least five readings over a period of more than fifteen minutes. The figures show no particular trend of the venous pressure with increasing anoxia. Figure 2 shows five of the patterns of venous pressure observed. Subject 16 showed a fairly

progressive fall. A similar pattern was seen in only four other individuals (3, 11, 13, 20) in this group (28 per cent). The remainder displayed a response similar to one of the upper four patterns in the figure. There was no predominance of any one pattern.

In the seven subjects (37 per cent) who showed a "circulatory crisis" with anoxia, a terminal, precipitous rise in venous pressure was observed simultaneous

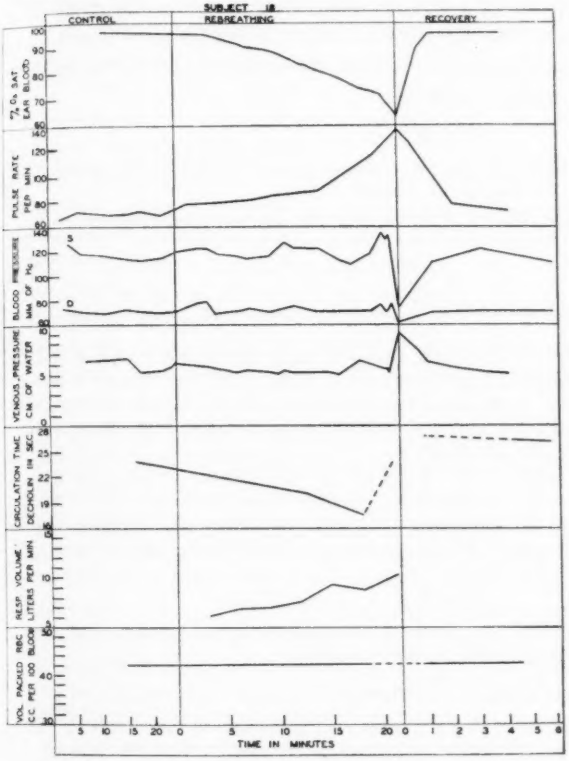

Fig. 1

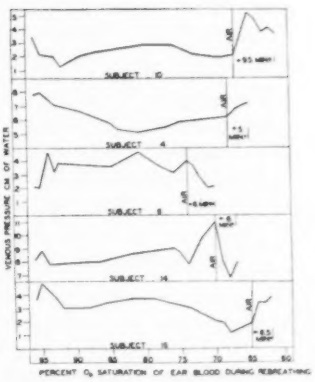

Fig. 2

Fig. 1. Circulatory measurements, respiratory volume, and volume of packed red blood cells during progressive anoxia in a normal white male, age eighteen years. The experiment was terminated because of syncope ("fainter").

Fig. 2. Direct venous pressure in five subjects during progressive anoxia. The vertical line labelled "air" at the right end of each chart indicates the point at which the subject was again permitted to breathe room air. The time during which venous pressure determinations were continued during the recovery period is also indicated on each chart.

with the onset of this crisis (table 1, subjects 2, 5, 8, 13, 14, 15, 18). A similar response has been observed in anoxic dogs (7).

The circulation time from the right antecubital space to the tongue was shortened in eighteen subjects during anoxia (table 2). In all but two (subjects 5, 13) this time was shorter when the oxygen saturation of the ear blood was 75 per cent

than when it was 85 per cent. Compared to the control, the greatest reduction when the oxygen saturation was 85 per cent was 7.2 seconds (subject 15); when the oxygen saturation was 75 per cent it was 13.0 seconds (subject 6).

In twelve subjects the circulation time could be ascertained in all four predetermined periods (table 2). In these twelve the average time during the control period was 19.2 seconds; with an oxygen saturation of 85 per cent and 75 per cent it was 16.3 seconds and 12.9 seconds respectively; and in the stage of recovery it was 21.7 seconds. The differences in these averages were found to be statistically significant except when the mean of the control period was compared to the mean of the recovery period.

TABLE 1

The venous pressure in centimeters of water in eighteen normal subjects during acute progressive anoxia

The oxygen saturation was measured in the ear blood by the photocell oximeter

O_2 SAT.	SUBJECT																		MEAN
	2	3	4	5	6	7	8	9	10	11	13	14	15	16	18	19	20	21	
%																			
Control (96)	6.1	8.1	7.7	8.5	2.1	2.6	7.6	5.1	3.5	3.3	3.5	8.1	5.5	3.5	5.9	8.4	6.6	6.9	5.7
95–92		8.0	7.5	10.0	3.5	3.1	7.9	5.7	2.2	2.5	3.6	8.3	6.2	3.8	5.6	8.3	7.3	7.2	5.9
91–87	6.8	8.2	6.4	9.3		2.9	7.2	5.4	2.4	3.3	3.3	8.0	5.8	3.0	5.3	8.5	7.3	7.2	5.9
86–82		7.6	5.6	8.8	3.5	3.2	7.0	5.6		1.6	2.7	8.6	5.6	3.5	5.2	9.6	5.9	7.2	5.7
81–77	7.6	6.6	5.4	12.2	4.1	5.0	7.2	5.1	2.8		2.7		5.3	3.6	5.1	9.1	4.1	6.7	5.8
76–72	12.5	6.8	5.8		3.2		7.8		2.5	2.6	3.0	8.5	4.8	3.0	5.9	10.4	4.2	7.6	5.9
71–67			6.2				7.4	6.0	2.3		2.8	10.4	4.8	1.2	5.3		3.4	8.1	5.3
66–62							9.4	6.4			3.4		8.4	1.8	9.4		3.8	8.1	6.3
Recovery (96)	9.5	6.8	7.0	8.2	2.3	3.9	7.5	5.5	4.3	2.7	3.3	7.3	5.2	3.6	5.5	8.6	6.1	7.0	5.8

TABLE 2

The right arm to tongue circulation time (decholin) in eighteen normal subjects before, during and after progressive anoxia

O_2 SAT.	SUBJECT																		MEAN
	2	3	4	5	6	7	8	9	10	12	13	14	15	16	18	19	20	21	
Control (96%)	25.4	22.5	19.0	24.4	26.2	13.4	10.0	22.8	23.3	24.0	14.0	14.3	29.2	17.0	23.0	24.0	19.0	14.6	20.3
85%	24.0	21.5	18.6	17.4	20.8	10.8	9.1	17.3	16.8	22.2	8.7	11.8	22.0	15.0	20.0	16.8	16.6	12.8	16.8
75%	B	15.0	B	20.2	13.2	B	8.2	14.6	16.2	B	10.2	8.6	18.6	14.5	17.5	11.2	14.0	11.7	13.8
Recovery (96%)					22.2	17.6	10.6	22.3	18.8	32.2	16.0	16.5	32.2	20.9	26.5	26.5	23.0	15.0	21.4

B = blank—the subject was unable to taste the injected drug.

DISCUSSION. The incidence of "fainting" and of "non-fainting" reactions in this small group is comparable to the results of Schneider's studies (8). He found 47 per cent in the first category, and 53 per cent in the second, compared to an incidence of 37 per cent and 63 per cent respectively in the present series. The usually accepted explanation for the circulatory crisis seen in some subjects is a failure of the peripheral vasomotor mechanism. The abrupt rise in the peripheral venous pressure that invariably accompanies such a reaction suggests rather that acute failure of the right ventricle is the principal cause (9).

The considerable shortening of the circulation time observed, in contrast to the results of Levy, Barach, and Bruenn, may be attributable to the different techniques used. The latter determined the rate of circulation before and at the end of a period of twenty minutes during which the subjects breathed a mixture of 12 per cent oxygen and 88 per cent nitrogen. It is possible that when the anoxia is not progressive, compensations may occur after twenty minutes which restore the rate of the circulation to its control value.

The average circulation time during the control period in our eighteen subjects was 20.3 seconds. In ten it was above this average, and in one its duration was 29.2 seconds. The usually accepted limits for circulation time determined by the decholin method is 10 to 16 seconds, and the average time in normal, basal subjects is 13 seconds (10).

The technique used in the present series differed from that used in others in that the needle was placed in the vein after the induction of local anesthesia. Further, the needle was left in situ and the subject was recumbent for at least 15 minutes before the first determination. The experiments were done late in the afternoon.

In order to explain these slow rates in normal subjects some further experiments are in progress, the results of which indicate that the whole matter of circulation time must be reinvestigated with a consideration of such variables as the posture of the subject, the position of the arm with respect to the thorax, the size of the needle, the effect of repeated venipuncture, the pulse rate, the blood pressure, and others. From a few preliminary experiments it appears that a simple venipuncture of itself can shorten the rate of the circulation from the arm to the tongue by as much as five seconds. If this puncture is accompanied by much trauma to the vein the circulation time may be greatly prolonged, possibly from reflex vasoconstriction as well as from venospasm caused by the direct stimulus. A precise explanation for the slow rates observed is lacking, but since the majority of these checked quite closely with the rate of the circulation determined at the end of the experiment after the subject breathed room air for a brief interval (table 2), the relative changes observed during anoxia appear valid.

In four instances (table 2, subjects 2, 4, 7, 12) the bitter taste was not perceived by the subject when the oxygen saturation of the ear blood was 75 per cent. Since anoxia is known to depress the acuity of various senses, it is probable that in these subjects the end organs of taste or the cerebral receptive centers failed to respond. If this depression occurred in the other experiments but to less degree, the figures on the circulation time are too high, and the rate is increased even more than indicated by these experiments.

CONCLUSIONS

In nineteen young, healthy male subjects, acute progressive anoxia, induced by rebreathing, had the following effects:

1. The venous pressure showed a variable response. In four subjects it progressively decreased. In seven subjects who fainted during the rebreathing,

the venous pressure rose precipitously just before syncope, suggesting failure of the right ventricle. In all cases the venous pressure was restored promptly to normal by permitting the subject to breathe room air.

2. The circulation time from the right arm to the tongue was decreased in all subjects. This decrease was statistically significant. The rate of circulation was normal or slightly slower in some cases as soon as the oxygen saturation of the blood was restored to the control level.

The authors are indebted to Riedel-de-Haen, Inc. for the supply of decholin sodium used in these studies.

REFERENCES

(1) Schneider, E. C. and D. Truesdell. This Journal **71:** 90, 1924.
(2) Hooker, D. R. and J. A. E. Eyster. Bull. Johns Hopkins Hosp. **12:** 274, 1908.
(3) Levy, R. L., A. L. Barach and H. G. Bruenn. Am. Heart J. **15:** 187, 1938.
(4) Moritz, F. and D. von Tabora. Deutsch. Arch. f. klin. Med. **98:** 475, 1910.
(5) Winternitz, M., J. Deutsch and Z. Brull. Med. Klin. **27:** 986, 1931.
(6) Millikan, G. A. Personal communication.
(7) Sands, J. and A. C. DeGraff. This Journal **74:** 416, 1925.
(8) Schneider, E. C. and D. Truesdell. This Journal **55:** 233, 1921.
(9) Wiggers, C. J. Ann. Int. Med. **14:** 1237, 1941.
(10) Tarr, L., B. S. Oppenheimer and R. V. Sager. Am. Heart. J. **8:** 766, 1932.

THE REPUTED RESERVOIR FUNCTION OF THE SPLEEN OF THE DOMESTIC FOWL

PAUL D. STURKIE

From the Alabama Agricultural Experiment Station, Auburn

Received for publication October 14, 1942

It was demonstrated by Barcroft (1) that the spleen of mammals serves as a reservoir of erythrocytes and that in times of emergency it expels the reserve cells into the general circulation. This function has been ascribed, also, to the avian spleen by Harmon *et al.* (2). They determined the blood hemoglobin of seven adult fowls before and after asphyxia with the spleen intact and after splenectomy. The birds were placed in a darkened coop and allowed to remain undisturbed for one hour after which time the first blood samples were taken from the wing veins. Immediately after taking the first sample, "asphyxia was induced by pinching the trachea from the outside until the bird went limp." The second sample was then taken quickly from the opposite wing vein. Existence of the reservoir function of the spleen was considered proven if the blood sample following asphyxia (second) showed an increase in hemoglobin over the sample before asphyxia (first) when the spleen was intact, and if no increase in the second sample over the first was observed after removal of spleen. The determinations were based, apparently, upon only one sample per bird and were made by means of a Dare hemoglobinometer. The figure reported represented an average of ten readings of the same sample. Later, Harmon (3) reported the presence of significant hemoglobin reserves in the spleens of young cockerels, immature pullets and mature hens (laying hens) but not in cocks and broody (non-laying) hens.

The reliability of Harmon's data may be questioned because it has been shown that the Dare hemoglobinometer is not suitable for the determinations on chicken blood. Moreover, the structure of the spleen in birds differs from that of mammals in characteristics unfavorable to the rapid and regulated expulsion of erythrocytes. These facts indicated the need for further study on the problem.

METHODS. In the following experiment, the procedure employed in taking the blood samples was not different from that of Harmon *et al.* except that instead of inducing asphyxia immediately after taking the first sample, an interval of approximately one hour elapsed before asphyxia was induced, after which time the second sample was taken. During this period, the birds were kept in a darkened coop which afforded no opportunity for disturbance. This change in procedure, it is believed, should have no adverse effect on the spleen in exerting its alleged reserve function. The samples were taken at weekly and bi-weekly intervals. All of the birds were laying before splenectomy and most of them continued to lay after splenectomy.

A number of methods have been employed in making hemoglobin determinations on fowl blood, and considerable variation in the values thus obtained resulted. The most reliable one is the Newcomer acid hematin method as modified

by Schultze and Elvehjem (5). This modification eliminates the turbidity of solution produced by the nucleated red cells of chicken blood, which is a common source of error for the unchanged method. The procedure followed in this study varied from that of Schultze and Elvehjem in that after dilution of the blood sample (0.01 cc.) with 5 cc. of 0.4 per cent NH_4OH, an equal amount of HCl, of appropriate dilution, was added. The hemoglobin of the acid solution was then determined with a photoelectric colorimeter. The results obtained are in close agreement with those of Schultze *et al.* (6).

Splenectomy was performed by making an incision on the right side of body between the last vertebral ribs. After blunt dissection and manipulation of the viscera, the spleen was exposed. A pair of cup-shaped forceps which could be extended around and under the organ was used. A loop of thread, slipped over the forceps and under the spleen was drawn together, thus ligating all blood vessels attached to it. The organ was then enucleated with fingers. Of a total of 26 birds, four died as a result of the operation. Blood samples were taken at six weeks to two months after the operation.

Results. The hemoglobin determinations for the various hens before and after splenectomy are shown in table 1, and the analysis of variance of these values is presented in table 2.

Before splenectomy. The results are based upon a total of 44 hemoglobin determinations for each of the samples before and after asphyxia, or upon four paired samples per hen. The means for the samples before and after asphyxia (table 1) are 8.90 and 9.12 grams per 100 cc. of blood. That the difference in these means is not significant is revealed by the insignificant mean square 1.08, for before and after asphyxia (table 2). When the sources of variation are segregated, it is observed that a significant portion of the difference in the above means is due to the variances between samples per hens and between hens. The interaction, which is not significant, indicates that the hens responded in a similar manner before and after asphyxia.

Of the 44 determinations after asphyxia, 26 showed a slight increase, in most cases less than 10 per cent, over the first samples, 16 showed a decrease and two of them were unchanged.

After splenectomy. The difference in the means of the samples before and after asphyxia (9.03 and 8.95 grams per 100 cc., respectively) is not significant, while the variances between hens and between samples per hen are significant. The interaction was omitted here because of unequal frequencies in some of the classes.

Of the 42 determinations after asphyxia, 24 represented a decrease in amount of hemoglobin over the first sample, 14 represented an increase and four were unchanged.

Hemoglobin determinations on first and second samples with spleen intact and no asphyxia. These analyses were made in order to determine the variation in hemoglobin between first and second samples of blood per hen taken under conditions in which the splenic reserve supposedly does not function. The samples were obtained from the same eleven hens (table 1) which were used for the splenic reserve study. An interval of one hour elapsed between collection of first and

TABLE 1

Hemoglobin in grams per 100 cc. of blood

HEN NO.	WITH SPLEEN INTACT		AFTER SPLENECTOMY		HEN NO.	WITH SPLEEN INTACT		AFTER SPLENECTOMY	
	Before asphyxia	After asphyxia	Before asphyxia	After asphyxia		Before asphyxia	After asphyxia	Before asphyxia	After asphyxia
1	9.90	11.05	9.55	9.55	7	11.50	12.60	9.90	9.25
	11.50	11.80	10.50	10.35		9.80	10.00	10.60	9.80
	10.00	10.35	11.20	10.35		10.30	9.80	10.50	11.00
	9.55	9.80	10.55	10.20		8.60	8.25	10.05	9.80
2	7.65	8.70	10.00	10.70	8	9.35	10.75	10.30	9.55
	9.25	8.80	9.30	8.75		9.75	10.85	10.30	9.75
	7.60	8.05	9.50	10.05		9.10	10.35	9.10	9.00
	8.25	8.05	9.55	9.10		9.55	9.30	9.55	9.25
3	8.60	8.10	6.90	6.50	9	9.30	9.80	9.05	9.55
	6.75	7.40	7.05	7.10		9.80	9.55	8.60	9.55
	6.60	7.15	9.55	9.55		9.30	9.20	8.05	8.05
	7.20	7.25	9.70	10.05		8.65	7.85	8.60	8.05
4	8.15	8.85	8.30	8.10	10	9.75	9.65	9.75	9.75
	9.55	9.15	7.60	7.00		10.00	10.75	10.05	9.15
	8.65	10.00	5.20	5.10		9.25	9.80	10.85	10.30
	8.80	9.05				9.95	9.75	9.55	10.20
5	9.05	9.05	7.55	8.10	11	8.70	9.10	6.40	6.50
	10.05	9.05	9.15	8.75		9.95	9.55	8.65	9.05
	7.40	7.65	10.15	10.05		9.10	9.35	9.40	8.70
	7.15	7.55				9.10	8.60	8.80	9.55
6	7.15	6.65	8.85	8.60					
	8.00	8.00	8.65	7.85	Means	8.90	9.12	9.03	8.95
	7.10	7.45	7.05	7.85					
	6.95	7.60	5.55	6.50					

TABLE 2

Analysis of variance of the hemoglobin values for hens before and after asphyxia, with spleen intact and after splenectomy

SOURCE OF VARIATION	BEFORE SPLENECTOMY			AFTER SPLENECTOMY		
	D.F.	Sums of squares	Mean squares	D.F.	Sums of squares	Mean squares
Total	87	135.02		83	151.55	
1. Before and after asphyxia	1	1.08	1.08	1	0.15	0.15
2. Between samples per hen	33	92.75	2.81*	31	87.45	2.82*
3. Between hens	10	89.83	8.98†	10	86.25	8.62†
4. Interaction	10	1.84	0.18			
5. Error	33	42.27	1.28	41	64.10	1.59

* Significant on 0.05 level.

† Significant on 0.01 level.

second samples, and during this period the birds remained at rest in a darkened coop. When they were removed for taking of samples, particular care was exercised in handling to prevent undue excitement.

The results are based upon 31 determinations each for the first and second samples, or in most cases, upon 3 paired samples per hen, and these were taken at weekly intervals. The mean hemoglobin in grams per 100 cc. for the first sample was 8.80 and for the second sample 9.02. The difference in these means is nearly the same as the difference between the means before and after asphyxia with spleen intact and is not significant. The variances between the different hens and between samples per hen are significant, as was true in the previous analysis.

Discussion. These results, contrary to those of Harmon *et al.*, reveal that asphyxia does not stimulate the spleen of the fowl to expel erythrocytes into the general circulation, as it does the spleen of mammals. It is known that the high degree of contractility of the mammalian spleen plays an important rôle in the expulsion of blood into the main circulatory channels (Barcroft, 1; Klemperer, 4), and that such contractability is dependent upon the thick muscular capsule and prominent trabeculae of the organ. The spleen of birds has a thin capsule with few muscle fibers and no true trabeculae (Klemperer, 4) and is capable of contraction only to a slight extent. From anatomical considerations, therefore, it is not surprising to find the splenic reservoir function absent in the fowl.

SUMMARY

1. No evidence was found for the existence of a reservoir function of the spleen in the domestic fowl. The means for the blood samples before and after asphyxia when the spleen was intact were 8.90 and 9.12 grams of hemoglobin per 100 cc. of blood. The means for the samples after splenectomy were 9.03 and 8.95 grams respectively. The differences in these means are not significant.

2. The means for the first and second samples (no asphyxia) with the spleen intact were 8.80 and 9.02 grams of hemoglobin respectively per 100 cc. of blood. These means are nearly the same as the means for the samples before and after asphyxia before removal of spleen, and are not significant.

REFERENCES

(1) Barcroft, J. Lancet **1:** 319, 1925.
(2) Harmon, I. W., E. Ogden and S. F. Cook. This Journal **100:** 99, 1932.
(3) Harmon, I. W. Poultry Sci. **15:** 53, 1936.
(4) Klemperer, P. Handbook of hematology, III. p 1591. Paul B. Hoeber Inc., New York, 1938.
(5) Schultze, M. O. and C. A. Elvehjem. J. Biol. Chem. **105:** 253, 1934.
(6) Schultze, M. O., C. A. Elvehjem, E. B. Hart and J. G. Halpin. Poultry Sci. **15:** 9, 1936.

REGIONAL RELATIONSHIPS OF RATE OF WATER LOSS IN NORMAL ADULTS IN A SUBTROPICAL CLIMATE[1]

GEORGE E. BURCH AND WILLIAM A. SODEMAN

From the Departments of Medicine and Preventive Medicine, School of Medicine, Tulane University and Charity Hospital of Louisiana, New Orleans

Received for publication October 15, 1942

It is well known from ordinary methods of clinical examination that there are variations in the rate of sweating from skin surfaces from different portions of the body. Observers have made attempts to measure these differences quantitatively. In most instances the methods employed were inaccurate. Galeotti and Macri (1) found the rate of insensible perspiration to vary from 60.1 mgm. per 10 sq. cm. of skin per hour of the palm to 10.6 mgm. on the medial region of the abdomen with the skin areas of other regions of the body having rate varying between these extremes. Several years later Kuno and his associates (2) measured the rate of insensible perspiration from the same areas and found essentially the same values. These and other values have been discussed by Kuno (2) and need not be presented in detail again.

The studies to be reported were made in order to determine the rate for a subtropical climate and also to learn whether or not there were any differences due to season when the subjects were studied under constant laboratory conditions.

METHODS AND MATERIALS. The measurements were conducted in an air-conditioned room, maintained at 75°F. ± 1° and relative humidity 50 percent ± 2 percent as a comfortable environment. To make the room hot and humid in order to stimulate visible sweating the temperature and relative humidity were increased to 105°F. ± 2° and 75 percent ± 2 percent respectively. There were no perceptible currents of air at any time. The flow of air was less than 20 feet per minute. The room was so designed and decorated to reduce psychic disturbances to a minimum (3). The subject did not have any contact with the observers and the greater part of the apparatus, once the small cups (fig. 1) had been sealed to the skin to pick up the water.

The method, previously described (4), consists essentially of conducting dry oxygen from a supply tank into chambers enclosing the digits or area of skin. There the water from the surface of the skin is vaporized and conducted through aluminum coils where it is trapped by freezing. The amount of water lost during a known period of time is determined from the differences in weight of the coils before and after the water is condensed. The chambers used to enclose the finger and toe tips are more or less similar to those previously illustrated (4). The brass chamber used for collecting the water from surfaces of skin such as on the forearm or abdomen is shown in figure 1. It consists of a chamber *c*, into which the dry oxygen enters. This oxygen then enters chamber *e* through 10

[1] The studies were supported by a grant from the Rockefeller Foundation.

openings, *d* radially placed in order to ensure thorough distribution. In *e* the dry oxygen collects the water from the surface of the skin. The water laden oxygen then flows through the large orifice, *b*, and through tubing, *a*, to the collecting coil, where the water vapor is condensed and weighed. The chamber is light and insures an even distribution of the dry oxygen with adequate collection of the water from the skin. The edges of the chambers which are resting on the skin can be shaped to conform with the part to be covered. The chambers are sealed to the skin with a water soluble rubber cement (Flex-O-Fix) which dries within 15 minutes without contracting and tugging the skin. Most of the cements tried pulled the skin and interfered with the underlying circulation.

The studies were conducted as follows: The subject entered the observation room which had already had its atmosphere adjusted to a temperature of 75°F. and a relative humidity of 50 percent. The patient removed all of his clothes except his underwear, entered a comfortable bed with an inner spring mattress and covered with cotton sheets or a woolen blanket, to suit his comfort. The chambers enclosing the parts were sealed in place and after a period of approximately 45 minutes the water lost from the enclosed areas of skin was measured.

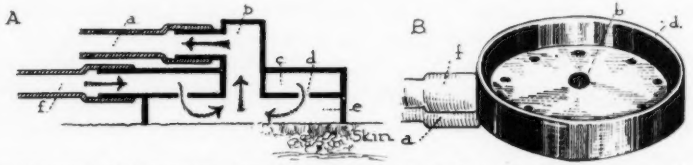

Fig. 1. Diagram of the metal chambers that were sealed to the skin to collect the water of sensible and insensible perspiration. They were built to cover variable areas of skin, usually 5 or 10 sq. cm.

The water was collected continuously throughout the entire period of study. By turning stopcocks and diverting the water laden oxygen from one collecting coil into another, the water loss could be separated into samples of 15 minute periods. Two or three collections of 15 minute periods each were made and then without the subject's knowledge the thermostat and humidistat were readjusted and the room temperature and relative humidity increased to 100°F. and 75 percent respectively. In about 15 minutes the atmospheric conditions of the room reached these new levels. During this period of change and for 30 minutes after the new level was reached the water loss from the areas of skin was measured so that at least two 15 minute collections were made with the room hot and humid.

The rate of water loss from the skin was measured during the winter and early spring months (January to March inclusive)[2] and again during the hot and humid summer months (July to August) in New Orleans. The conditions of the experimental room were the same during both seasonal periods. As many of the same subjects were used during each season as were available.

[2] Average temperature and relative humidity for New Orleans for the period of January to March, 1942, inclusive, were 55.1°F. and 68.6 per cent respectively and for July and August 1942, 83.0°F. and 80.3 per cent.

No attempt was made to make the determinations with the subjects in a post-absorptive state. They were advised to eat a light breakfast or lunch and were then studied about 2 to 3 hours later so that digestion was at a low ebb during the observations.

The studies were conducted on 46 normal adults, 14 of whom were females and 32 males. Forty-two were white and 4 negro. They varied in age from 20 to 53 years, only one being above 45 years. Thirty-seven subjects were studied during the winter season and 12 during the summer.

The 17 areas of skin studied were that over the right index finger tip, right second toe tip, right forearm (volar surface), external surface of the right forearm, epigastrium, anterior surface of the right thigh, posterior surface of the left thigh, area over the right cheek, forehead, right axilla, left axilla, posterior surface of the right hand, posterior surface of the left leg, right flank, plantar surface of the heel of the right foot, plantar surface of right foot in the region of the heads of the metatarsals, the mid-plantar surface of the right foot, and the palm of the right hand. Three or four areas were studied simultaneously. When the observations were repeated in the summer months only a few of these areas of skin were selected for study.

RESULTS. A total of 691 separate 15 minute determinations were made on the 46 subjects.

The rate of water loss from areas of skin was expressed in milligram per 10 sq. cm. of skin area, per 15 minutes. These units will not be repeated, only the numerical values will be given.

The rate of water loss was found to vary markedly from area to area, from patient to patient and from time to time in the same patient. Figures 2 and 3 summarize the mean and extreme values for 17 different areas of the body.

The mean and extreme rates of insensible and sensible water loss respectively for the various parts of the subjects studied during winter and early spring months are shown in figure 2.

The rates of water loss determined in subjects during the summer months for the right index finger tip, right second toe tip, right forearm, middle of the forehead and mid-epigastric region are summarized in figure 3. It can be seen that the values obtained in the summer months are approximately the same as those measured during the winter months. The only part that appears to show a significant difference is the volar surface of the forearm, the rate being greater during the summer. With a study of many more subjects, this difference may be lost.

The individual variations are great and the variations in the same individual from one 15 minute period to the next may be large. Figure 4 illustrates these variations for two parts, namely, the right index finger tip and the right forearm. The variations from subject to subject are greater when he is in a hot and humid room than when the room is comfortable. Figure 5 illustrates variation from one 15 minute period to the next over a period of one hour when the subject rested in a comfortable room. There are no prolonged studies for a hot and humid room.

The rate of water loss from the right index finger tip, volar surface of the right forearm and the anterior surface of the right thigh of one subject was studied, in

which the temperature and relative humidity were raised from 75°F. and 50 percent respectively to 95° and 75 percent, to 100° and 75 percent. The atmospheric conditions were maintained sufficiently long for the water loss to reach a level before the conditions were changed. When the temperature and relative humidity were increased to 95° and 75 percent, an increase of 20° and 25 percent respectively, the rate of water loss is increased slightly and a new level is reached

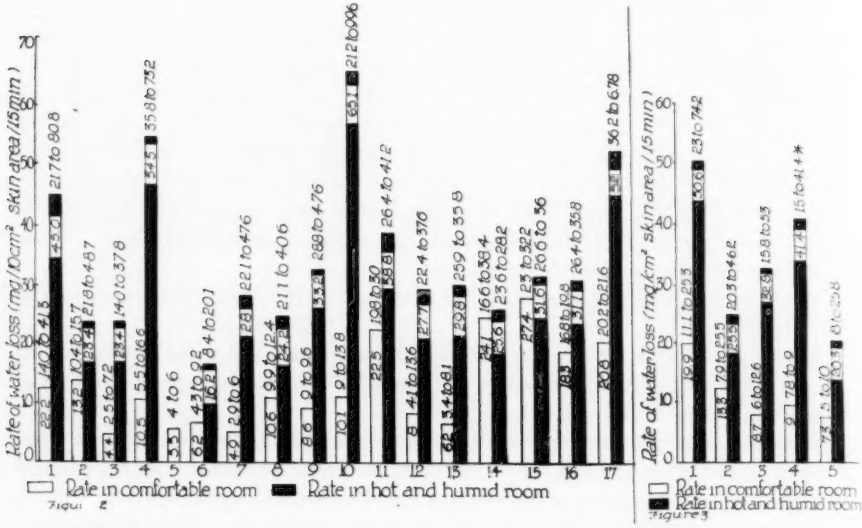

Fig. 2. The mean and extreme values for the rate of water loss from 17 different skin areas of 37 normal adults obtained during the months of January through March. The parts studied are represented as follows by the numbers along the abscissa: 1 = Right index finger tip, 2 = right second toe tip, 3 = volar surface of right forearm, 4 = middle of forehead, 5 = mid-epigastrium, 6 = anterior surface of the right thigh, 7 = posterior surface of the left thigh, 8 = right cheek, 9 = right axilla, 10 = left axilla, 11 = palm of the right hand, 12 = posterior surface of the right leg, 13 = right flank, 14 = plantar surface of the heel of the right foot, 15 = ball of the right foot, 16 = mid-plantar area of the right foot, 17 = lateral surface of the right arm.

Fig. 3. The mean and extreme values for the rate of water loss from 5 skin areas of 12 normal adults obtained during the months of July and August. * = variation in only one patient. The parts studied are represented by numbers along the abscissa and are the same as in figure 2.

(fig. 6). When the temperature is further increased only 5° and the relative humidity is kept at 75 percent there was a marked increase in the rate of water loss in all three parts. The measurements were not continued long enough to establish a new baseline.

Discussion. The water lost with the subject in a comfortable atmosphere was termed insensible, using the usual concept of terminology. The rate varied

considerably with the area of skin. In descending order it was as follows: hands, feet, head, arms, legs and trunk. The sensible water loss, stimulated by a hot and humid environment also varied with the area. The rate was greater for the finger tips, axillae and forehead. Although the insensible rate was slow from the skin of the trunk, the sensible rate became marked. The percentage increase was as great or greater from the skin of the arms, legs, and trunk, as from the finger and toe tips. The marked rate of sensible water loss from the

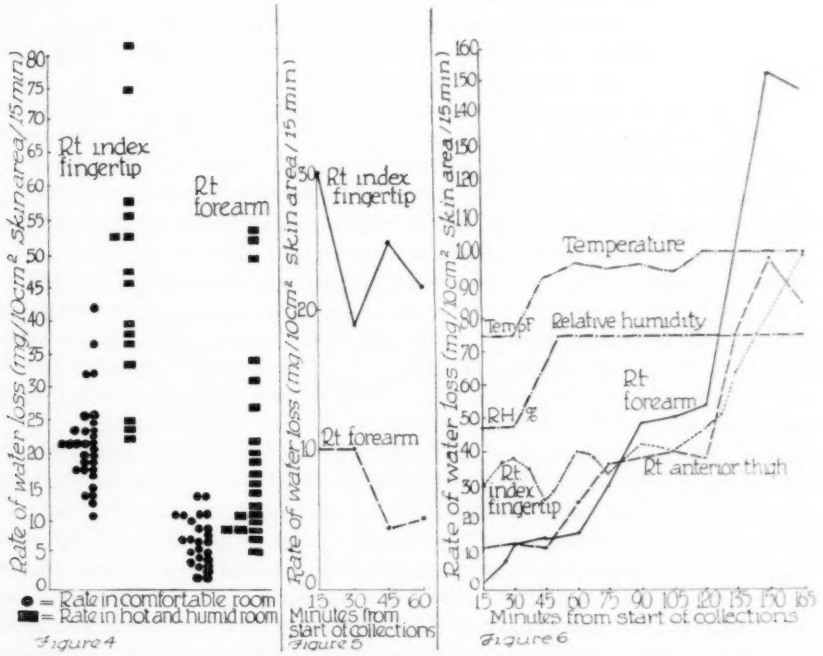

Fig. 4. Variations among individuals in the rate of water loss from two selected skin areas.

Fig. 5. Variations during one hour of the rate of water loss from two selected skin areas in one subject.

Fig. 6. The effect of variations of room temperature and humidity on the rate of water loss from three skin areas of one subject.

skin of the forehead and left axilla shown in figure 2 is due to the unusually rapid rate in one of the subjects studied. It is likely that if a larger number of subjects were studied such discrepancies would be smoothed out.

It would have been better to have measured all of these parts simultaneously rather than in groups of 3 or 4 parts at a time. The apparatus used in these studies was not constructed for so large a number. Kuno and his associates (2) used small celluloid chambers enclosing discs of blotter-paper to absorb the water that escaped to the surface of the skin. A larger number of these were

sealed on many different areas of the skin more or less simultaneously. The blotter-paper was weighed before and after absorbing sweat in order to determine the rate of water lost during a given time. Although such a method lends itself for easy measurement of many areas simultaneously, it is open to numerous errors and is relatively inaccurate. This probably accounts for the fact that the values found by Kuno and his associates are approximately half as great as the values found in these studies. Of course, the differences in race of the subjects, climatic conditions, and laboratory conditions (2) must have contributed to these differences. The relationships of the regional variations of water loss in these studies are essentially the same qualitatively as those reported by Kuno and his associates (2) and Galeotti and Macri (1).

The marked variations in the rate of sensible perspiration from subject to subject is not surprising (fig. 4). Everyone is acquainted with the variations of sweating in different individuals from his own casual inspection. Such marked variations in both insensible and sensible perspiration have been reported by many others (2). The factors that influence sweating that might be related to such variations were discussed in a previous report (5).

In a previous report (5) data indicated that there might be seasonal variations in insensible water loss from the skin measured under constant laboratory conditions. The present studies do not support such a contention. Since it is so difficult to control all factors that influence sweating so that the season is the only variable it would be preferable not to draw any conclusions from these or similar studies as yet.

As illustrated by figure 6, sensible perspiration in a resting adult does not become marked until the environmental temperature and humidity become very unfavorable for loss of heat from the body. When the room temperature was 95°F. and the relative humidity 75 percent and with the subject resting quietly in bed, the subject felt relatively comfortable although he knew he was in a very warm room. Sweating increased, but not markedly. These observations are in keeping with those of DuBois and his associates (6) who noted that under more or less similar conditions without muscular activity heat loss by radiation might be sufficient to maintain the normal thermal level of the body. When the room temperature was increased to 100°F. and the relative humidity maintained at 75 percent, there was an immediate and marked increase in the rate of sweating. Under such conditions heat was passing from the atmosphere to the body and convection currents (less than 20 ft. per min.) were of no value. The only possible source of heat loss from the body was through evaporation. This could not be marked because of the high relative humidity. Therefore, sensible perspiration which would be of relatively little value was stimulated and sweat poured. Such sweating, if prolonged, may be detrimental since it can disturb markedly the water and electrolyte balance. If such sweating continues over a period of a half to one hour or so the subject may show ill effects. At the temperature of 95°F. and a relative humidity of 75 percent and with the subject resting quietly, the rate of heat production and heat loss were about at equilibrium. Should production have increased as with mild exercise, body tempera-

ture would tend to rise and sweating result. When heat loss was interfered with by increasing the room temperature and humidity, sweating became marked even with the subject at rest. Under conditions of high temperature and humidity when heat loss cannot be rapid, such as happens in sub-tropical and tropical climates, exercise or heat production should be kept as low as possible.

SUMMARY

In a study of 17 different areas of skin of 46 normal adults it was found that there is a marked regional variation in the rate of sweating. The most rapid rates of insensible perspiration are from the hands, feet, forehead and cheeks. The skin of the trunk, arms and legs has relatively slow rates of insensible water loss. There are marked variations in the rate of insensible water loss for the same area from subject to subject and from time to time in the same subject. These marked variations result in overlapping of values for the various areas.

Similarly, there are marked variations in the rate of sensible perspiration stimulated by a hot and humid environment. The rate of water loss increased often to a greater extent from those areas which showed relatively little insensible sweating when sensible sweating occurred, than from the areas which showed the largest rates of insensible water loss.

There is no definite evidence of difference in the rate of insensible or sensible water loss during winter or summer months when the measurements are made under constant laboratory conditions.

The rate of water loss from the skin of a subject resting quietly in bed is not materially increased when the temperature and relative humidity are increased from 75°F. and 50 percent respectively to 95° and 75 percent. When the temperature is further increased to 100°F., sweat literally pours. In a humid subtropical and tropical environment, when heat loss is interfered with, muscular exertion should not be maintained for prolonged periods of time.

Acknowledgment. We wish to express our appreciation for the excellent technical assistance and keen interest of Mr. G. Morgavi, who participated in these studies and constructed the apparatus.

REFERENCES

(1) Galeotti, G. and N. M. Macri. Biochem. Ztschr. **67:** 472, 1914.
(2) Kuno, F. The physiology of human perspiration. J. & A. Churchill, London, 1934.
(3) Newmann, C., A. E. Cohn and G. E. Burch. J. Clin. Investigation. In press.
(4) Newmann, C., A. E. Cohn and G. E. Burch. This Journal **132:** 748, 1941.
(5) Burch, G. E., A. E. Cohn and C. Newmann. Am. Heart J. **23:** 185, 1942.
(6) DuBois, E. Lane Medical Lectures; The mechanism of heat loss and temperature regulation. Stanford University Press, Stanford University, California, 1937.

EFFECTS OF INHALATION OF 100 PER CENT AND 14 PER CENT OXYGEN UPON RESPIRATION OF UNANESTHETIZED DOGS BEFORE AND AFTER CHEMORECEPTOR DENERVATION[1]

JAMES G. WATT[2], PAUL R. DUMKE AND JULIUS H. COMROE, JR.

From the Laboratory of Pharmacology, University of Pennsylvania

Received for publication October 16, 1942

Since the discovery of the aortic and carotid chemoreceptors, a large number of investigations have been performed upon these structures (for bibliography, see (13)). However there is still disagreement upon two points of fundamental importance. The first of these is the question of the existence of tonic activity. Some investigators (11, 8, 9) maintain that chemoreceptor reflexes are of great importance in the control of respiration under all conditions (normal as well as abnormal) while others (4) believe that these structures function chiefly as an emergency mechanism, of extreme importance during anoxemia, asphyxia, acidosis, and marked hypercapnia, but relatively unimportant in the control of normal breathing. The second point has to do with direct stimulation of the medullary centers by anoxia. Although experiments upon anesthetized animals indicate that anoxia usually stimulates the medullary centers only reflexly through the chemoreceptors, it has been reported (12) that anoxemia may stimulate the medullary centers directly if the anesthesia is sufficiently light. Since anesthesia may either intensify or depress chemoreceptor activity depending upon the nature and concentration of the anesthetic[3] we decided to investigate these problems on trained unanesthetized dogs.

METHODS. Mongrel female dogs of varying size and breed were trained to lie quietly upon a table while breathing through a moulded plaster mask reinforced with rubber and fitted with inspiratory and expiratory valves. All gases, including room air, were inhaled from identical Douglas bags attached to a 3-way stopcock on the inspiratory side. Minute volume of respiration was measured by passing the expired air through a gas meter and respiratory rate was measured by a pneumograph and tambour. The periods of inhalation of each gas were usually 6 minutes; when samples of arterial blood were drawn, these were col-

[1] This investigation was partly financed through the National Committee for Mental Hygiene from funds granted by the Committee on Research in Dementia Precox founded by the Supereme Council, 33° Scottish Rite, Northern Masonic Jurisdiction, U. S. A.

[2] Ellen Mickle Fellow of the University of Toronto.

[3] Chloralose (100 mgm./kilo intravenously) in the dogs used in these experiments depressed respiratory minute volume (average reduction 36 per cent) and arterial pO_2 (average reduction 25 mm. Hg) and raised arterial pCO_2 (average increase 5.2 mm. Hg). At the same time, the degree of anoxia and the amount of NaCN necessary to produce stimulation of respiration were lessened. Chloralose anesthesia therefore exaggerates the effects of chemoreceptor reflexes. Experience of other investigators (1) (6) (12) with different anesthetics has shown that the influence of anesthesia on chemoreceptor reflexes is too complicated to permit generalizations from experiments with one type and grade of anesthesia on one species of animal.

lected under oil from the femoral artery at the end of the 6 minute periods, heparinized, covered with melted paraffin and kept in ice until analyzed. Estimations of plasma pH were made with a closed glass electrode at 38°C. and CO_2 content and whole blood O_2 content and capacity were determined by the Van Slyke manometric method. Experiments performed during the summer months were carried out in an air-conditioned room at 70°F. A complete experiment consisted of a series of observations (inhalation of room air, 100 per cent O_2, room air, 14 per cent O_2, room air) until the responses were consistent. The same series of observations was repeated after denervation of the carotid and aortic bodies.

Carotid body denervations were done under ether anesthesia; the internal carotid and occipital arteries and all attached nerve tissue were divided between ligatures and the external carotids were stripped from the origin of the internal carotid to the origin of the lingual artery. Aortic body denervations were performed by Dr. Norman Freeman in the following manner: The dog was anesthetized by intratracheal insufflation of ether; through an incision in the right third intercostal space, all branches of the right vagus and recurrent laryngeal nerves to the heart and aorta were severed below the level of the origin of the latter nerve. All branches from the stellate ganglion to the right vagus were cut and one inch of this vagus was excised below the origin of the recurrent laryngeal. Great care was taken not to injure the right recurrent laryngeal nerve itself. Through a similar incision on the left side all branches of the left vagus inside the thorax were severed including the left recurrent laryngeal at its origin from the vagus, but the stripped left vagus trunk was left intact. This operation preserves one recurrent laryngeal nerve, one abdominal vagus trunk and a few of the fibers from the pressure receptors situated in the brachiocephalic artery, but interrupts all fibers from the aortic bodies. Complete chemoreceptor denervation was attested by the lack of respiratory stimulation from intravenous injections of NaCN or inhalation of low oxygen mixtures which previously had caused marked hyperpnea; incomplete aortic pressure receptor denervation was indicated by a return of blood pressures to the normal range within 2–4 weeks, after a temporary hypertension.

RESULTS. A. *Tonic activity of chemoreceptors during quiet breathing of room air.* This was investigated by substituting 100 per cent O_2 for room air while respiratory rate and minute volume were measured. We chose this procedure because the arterial blood of an animal breathing room air always shows some oxygen unsaturation, and if this is sufficient to set up tonic chemoreceptor impulses capable of stimulating respiration, inhalation of 100 per cent O_2 should result in a definite depression of respiration. The average results of 194 observations on seven trained normal dogs are shown in figure 1. The scatterings of the findings in each dog are shown in table 1. In each dog there was usually an immediate decrease in minute volume which was maximal at the end of the first minute; the magnitude of this depression varied from 11 to 31 per cent. Minute volume had returned to a normal level by the end of 3 minutes in 3 dogs and by the end of 6 minutes in another, but in 3 dogs respiration was still 10

TABLE 1

Effect of inhalation of 100% O_2 upon minute volume of respiration

INTACT DOG	TOTAL OBSERVATIONS	DECREASE IN MINUTE VOLUME	INCREASE IN MINUTE VOLUME	NO CHANGE IN MINUTE VOLUME
1	44	35	9	
2	49	33	12	4
3	14	12	2	
4	12	12		
5	47	46	1	
6	13	12	1	
7	15	15		
DENERVATED DOG				
1	6	2	4	
2	22	3	18	1
3	9	2	7	
4	6		5	1

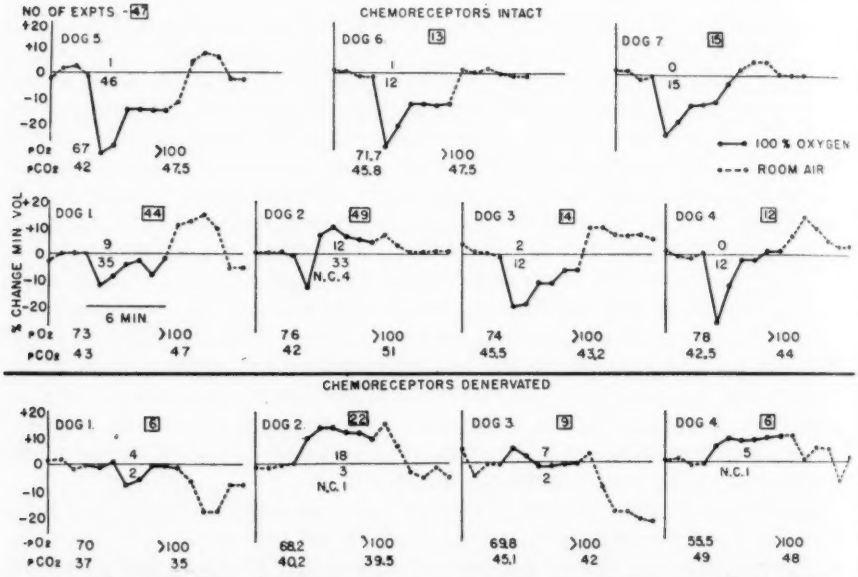

Fig. 1. Average effect of 100 per cent oxygen on respiration of the unanesthetized dog. Upper charts represent average percentage change in minute volume of respiration of normal unanesthetized dogs during inhalation of room air (broken lines), 100 per cent O_2 (solid lines) and then room air again; observations are recorded at minute intervals. The numbers in squares indicate the number of experiments performed upon each dog. The numbers just above the zero percent line represent the number of experiments in which respiratory minute volume increased, and those just below the line represent the number of experiments in which respiration decreased during inhalation of 100 per cent O_2 (N.C. = no change). Arterial oxygen tensions (pO_2) and arterial CO_2 tension (pCO_2) were determined upon femoral artery blood withdrawn at the ends of the control period and of the O_2 inhalation.

Lower charts show similar data upon dogs 1 to 4 after denervation of carotid and aortic bodies.

to 14 per cent below the control level at the end of the 6th minute of O_2 inhalation; in these, return to normal occurred promptly upon inhalation of room air. In 5 of 6 dogs in whom CO_2 tensions of arterial blood were measured, pCO_2 had increased 1.5 to 9 mm. (average 3.8 mm.) at the end of the O_2 inhalation.

The same procedures were repeated after chemoreceptor denervation in 4 of the 7 dogs (fig. 1 and table 1). (One dog died before operation, one died following carotid denervation and a third has not been operated upon as yet.) The immediate decrease in minute volume that occurred consistently when O_2 was inhaled by the intact dogs was lacking completely after the denervation.

Comment. Since all these animals usually showed an immediate depression of pulmonary ventilation when they were made to breathe 100 per cent O_2, and since chemoreceptor denervation entirely abolished this effect, it follows that some of their chemoreceptor units must have been tonically activated by the oxygen unsaturation normally existing in their arterial blood during quiet

TABLE 2

Arterial blood analyses—breathing room air

DOG	O_2 SAT.	pCO_2	pH	M.V. (AVERAGE)
1 Intact	95	43	7.40	2540
Denerv.	95.5	37	7.48	2500
2 Intact	95.5	42	7.40	2350
Denerv.	95.4	40.2	7.44	2700
3 Intact	94.0	45.5	7.37	2500
Denerv.	94.2	45.1	7.38	2800
4 Intact	95.0	42.5	7.35	2550
Denerv.	88.0	49.0	7.35	3400

breathing of room air at sea level. The interpretation of this finding, however, should be made with due regard for the following facts:

1. Denervation of the carotid and aortic bodies did not lead to a lower resting volume of pulmonary ventilation in any of these animals (table 2), or to any significant change in arterial gas content or pH except in dog 4, which we believe had some atelectasis. Hence it would appear that tonic chemoreceptor activity was not essential for the maintenance of normal respiratory activity in these animals breathing room air. The same conclusion is suggested by the tendency of breathing to recover during the inhalation of O_2 in 4 out of 7 intact dogs, and by the qualitative variations in the responses of the same animal from day to day. This is particularly evident in dogs 1 and 2 (table 1).

2. Inhalation of 100 per cent O_2 at sea level should cause a rise in arterial pO_2 from a normal of 70 to 90 mm. Hg to something above 600 mm. Hg, which is of course far beyond the physiological range. When these dogs were made to breathe mixtures low in O_2 we obtained evidence, confirmatory of a previous report (4), indicating that a change of 30 mm. Hg in arterial pO_2 (i.e., from 80 to

50 and back to 80 mm. Hg) causes practically no change in respiratory minute volume in the dog, but a further change of 10 mm. Hg (i.e., from 80 to 40 mm. Hg) causes definite hyperpnea and the reverse change (from 40 to 80 mm.) leads to abrupt depression of breathing. At the critical level, therefore, small changes in arterial pO_2 cause more marked alterations in chemoreceptor activity than do much larger changes in pO_2 at a normal or supernormal level. This supports the suggestion (13) that while some chemoreceptors are sensitive enough to be activated by the small degree of arterial unsaturation normally present, most of them come into action only at a definitely subnormal pO_2.

3. Even though some chemoreceptors show tonic activity in dogs at sea level, they do not appear to do so in man. It has been reported (15) that O_2 inhalation causes respiratory stimulation in healthy young adults, but the conclusion was based on the average obtained during a 15 minute period and no mention is made of the immediate effect. We investigated this point in 11 young adults. Of 19 experiments, the immediate effect of inhaling 100 per cent O_2 instead of room air was an increase in respiratory minute volume in 13, a decrease in 4, and no change in 2. The average of the 19 observations gave a 6 per cent increase. These findings suggest that tonically active, oxygen-sensitive chemoreceptors are the exception rather than the rule in normal man.

While the existence of chemoreceptors tonically activated by the degree of oxygen unsaturation normally present in unanesthetized dogs is definitely confirmed, no evidence was obtained bearing upon the question of similar activation by changes in CO_2, pH, or temperature (8, 14).

B. *Direct effects of anoxia on the respiratory center.* The same dogs were given 14 per cent O_2 to inhale for a 6 minute period using the same experimental technique described in A. The average results, before and after denervation, are shown in figure 2. In each of the 7 normal dogs, 14 per cent O_2 increased respiratory minute volume, the range being 17 to 29 per cent; in the 4 denervated dogs the initial effect was depression of respiration by 22 to 29 per cent. These results are similar to those reported in unanesthetized (2) and in anesthetized dogs (7). The absence of stimulation in the denervated dogs cannot be attributed to a failure of arterial pO_2 to fall, for the average arterial pO_2 (calculated according to Dill's data (5)) during inhalation of 14 per cent O_2 was 48 mm. Hg before denervation, 33 mm. Hg afterward. The first effect of anoxia upon respiration of these dogs was unquestionably depression because rate, depth of breathing, and respiratory minute volume were decreased in each of the 4 dogs and in every one of the 25 observations made with 14 per cent O_2 after the denervation. The depression began within the first minute and lasted about four minutes, after which the minute volume began to increase, though it was still 12 to 20 per cent below normal at the end of the six minute inhalation period. This apparent recovery was associated with restlessness and in several instances convulsive movements were observed, but it is noteworthy that the increase in breathing was due entirely to acceleration, often of the rapid, shallow type; depth was never increased and was usually decreased.

Comment. These findings are presented as additional evidence that the char-

acteristic respiratory response to anoxemia (prompt increase in depth of breathing causing a diminution in the ΔpO_2 between inspired air and arterial blood) is due to chemoreceptor reflexes. This conclusion is not vitiated by the fact that, in the denervated dog exposed to atmospheres low in oxygen, the primary respiratory depression may be followed by an increase in respiratory minute volume toward or above normal (2) (12). While we have not prolonged the low O_2 inhalations in our experiments beyond 6 minutes, we did notice, following the depressed period of 4 minutes, an unmistakable tendency for the minute volume to rise despite continued anoxia; this was due entirely to an increase in rate, depth being unaffected or decreased. Goldschmidt, Brewer, Daven-

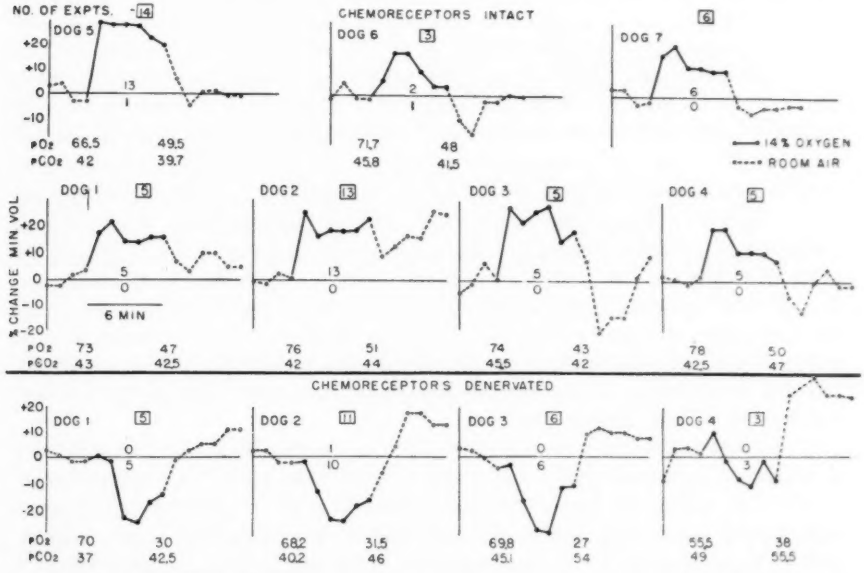

Fig. 2. Average effect of 14 per cent oxygen on respiration of the unanesthetized dog. Similar to figure 1 except that solid lines represent inhalation of 14 per cent O_2 in 86 per cent N_2.

port and Chambers (10) have analyzed the effects of prolonged anoxia in the surviving 3 of our 4 denervated dogs, and have found that minute volume eventually returned to normal and then usually rose above normal.

Several explanations might be offered for this delayed response other than regeneration of nerve fibers: *1.* Chemoreceptors elsewhere (e.g., in the coccygeal body) possessing much lower sensitivity to anoxia than those in the carotid and aortic bodies might be responsible; this is unlikely because this particular response appears to be depressed easily by anesthesia (12) while the carotid and aortic chemoreceptors are characterized by a high resistance to most forms of anesthesia (4).

2. As the oxygen saturation falls to lower levels upon prolonged exposure to

low oxygen, acid metabolites may accumulate in the respiratory center and so stimulate respiration. While there is no direct evidence favoring this explanation, it is in harmony with the views of those physiologists (9) who believe that respiration is controlled predominantly by the hydrogen ion concentration within the cells of the respiratory center. Since anoxia of this degree probably would result in accumulation of greater amounts of acid than under ordinary conditions, it might be pertinent to inquire why acidity in this instance increases only the rate of breathing, and why a latent period of 4 minutes must elapse before stimulant concentrations are reached. If acidity in general (rather than CO_2 specifically) is the characteristic stimulus to the respiratory center, it should act promptly and should affect both rate and depth, for excess CO_2 is known to increase both rate and depth very promptly whether given by inhalation or by direct injection into the respiratory center (3). Since in the case of anoxia the acid metabolites are formed presumably within the cells of the center, the question of relative rates of diffusion of acid and CO_2 should be irrelevant. It may be argued that anoxia by a dual mechanism is depressing while stimulating, and consequently the acid-mechanism is working at a disadvantage. However anoxia does not correspondingly alter the response of the center to CO_2 inhalation. Dumke, Chiodi and Schmidt (7) found in denervated dogs average increases in respiratory minute volume of 52 per cent during inhalation of 3.5 per cent CO_2 in O_2, and of 46.4 per cent on inhalation of the same CO_2 concentration in 10 to 12 per cent O_2; in all cases, both rate and depth were increased and the hyperpnea appeared promptly. If CO_2 is effective only by virtue of its acid properties, it would be rather remarkable that the response to it is not markedly reduced by a degree of anoxia that drastically alters the response to a direct increase in intracellular acidity.

3. A third explanation is suggested by the observations that this polypnea is abolished by decerebration (2) or by a slight increase in the depth of anesthesia (12), which also suggests a supra-tentorial origin. However, preliminary experiments upon decerebrated cats and dogs have occasionally shown acceleration of rate after an initial depression of depth and minute volume when 14 per cent O_2 was breathed after carotid denervation and vagotomy. Consequently the phenomenon is not necessarily dependent upon the higher centers.

CONCLUSIONS

In 7 unanesthetized dogs, inhalation of 100 per cent oxygen for 6 minutes led to a transient diminution in respiratory minute volume varying from −11 to −31 per cent. After denervation of the carotid and aortic bodies, oxygen inhalation produced no change or an increase in minute volume of respiration. Consequently some chemoreceptors in the dog must be continually activated by the usual degree of oxygen unsaturation of arterial blood at sea level. However experiments upon normal men failed to reveal evidence of similar tonic activity.

Inhalation of 14 per cent O_2 in unanesthetized dogs increased respiratory minute volume 17 to 29 per cent; after chemoreceptor denervation, initial depression of minute volume (22–29 per cent) was observed. However, unlike the

sequence in anesthetized dogs, in which respiratory depression is usually progressive until death, in the unanesthetized denervated dogs depression of depth and rate of breathing was succeeded by acceleration of rate. Therefore known chemoreceptor reflexes cannot be responsible for all the increase in rate during prolonged anoxia. The possible causes of this delayed response are discussed.

REFERENCES

(1) Beecher, H. K. and C. A. Moyer. J. Clin. Investigation **20:** 549, 1941.
(2) Bouckaert, J. J., C. Heymans and A. Samaan. J. Physiol. **94:** P4, 1938.
(3) Comroe, J. H., Jr. This Journal **133:** P243, 1941.
(4) Comroe, J. H., Jr. and C. F. Schmidt. This Journal **121:** 75, 1938.
(5) Dill, D. B., H. T. Edwards, M. Florkin and R. W. Campbell. J. Biol. Chem. **95:** 143, 1932.
(6) Dumke, P. and R. D. Dripps. Federation Proc. **1:** 150, 1942.
(7) Dumke, P., C. F. Schmidt and H. P. Chiodi. This Journal **133:** 1, 1941.
(8) Euler, U. S. Von and G. Liljestrand. Acta Scand. Physiol. **1:** 93, 1940.
(9) Gesell, R. Ann. Rev. Physiol. **1:** 185, 1939.
(10) Goldschmidt, S., G. Brewer, H. W. Davenport and A. H. Chambers. Personal communication.
(11) Heymans, C. and J. J. Bouckaert. Ergebn. d. Physiol. **41:** 28, 1939.
(12) Moyer, C. A. and H. K. Beecher. This Journal **136:** 13, 1942.
(13) Schmidt, C. F. and J. H. Comroe, Jr. Physiol. Rev. **20:** 115, 1940.
(14) Schmidt, C. F., J. H. Comroe, Jr. and R. D. Dripps, Jr. Proc. Soc. Exper. Biol. and Med. **42:** 31, 1939.
(15) Shock, N. W. and M. H. Soley. Proc. Soc. Exper. Biol. and Med. **44:** 418, 1940.

A STUDY OF THE EFFECT OF SPONTANEOUS VARIATIONS IN BLOOD PRESSURE UPON SPONTANEOUS VARIATIONS IN THE VOLUME OF THE FINGER TIP[1]

CHARLES NEUMANN

From the Hospital of the Rockefeller Institute for Medical Research, New York, N. Y.

Received for publication October 17, 1942

During the past few years reports from Burton (1), Hertzmann and Dillon (2) and Burch, Cohn and Neumann (3) have established the fact that small blood vessels undergo spontaneous variations in volume not related to any recognized effect of respiration or heart beat upon the peripheral circulation. Burch, Cohn and Neumann have described in detail some of these fluctuations as they occur in fingers, toes and pinnae. Records made with a pneumoplethysmographic technique showed rhythmic changes in volume which were classified in three general types and named alpha, beta and gamma waves. The alpha waves, representing the most rapidly recurring fluctuations had an average rate of 7.9 deflections per minute and average size of 14.5 cu. mm. per 5 cc. of finger tip.[2] The beta and gamma waves were larger and slower than the alpha waves and represented gradual changes whereas, in most instances, alpha deflections were completed within a few seconds. It is to be emphasized that none of these changes were regular in frequency or volume; each in turn, including the effects due to cardiac impulse and respiration, was imposed upon the next larger and slower "rhythm" to give a continuously changing record of the size of the part studied.

With the origin of these waves in doubt, one of the problems which arises concerns itself with their dependence upon the blood pressure. Steele (4) in his study of intra-arterial measurements of the blood pressure in human subjects noticed variations both of systolic and of diastolic pressures, independent of respiration and raised the question of relationship between fluctuations in pressure and those in the volume of peripheral parts. To settle this question simultaneous records have been made of the intra-arterial blood pressure and of alpha waves.

MATERIALS AND METHOD. The apparatus consists of two important parts, of which one is the sensitive pneumoplethysmograph of Turner (5) and the other the hypodermic manometer of Hamilton, Brewer and Brotman (6). Both these devices, together with the necessary camera and timers, were assembled in a single unit which could easily be transported to the bedside. All of the working parts were enclosed in a light-tight case to permit the subject to be in a lighted room at the time of the study.

The hypodermic manometer was built in accordance with the design of Hamil-

[1] This is the tenth paper reporting the results of studies of the small blood vessels and related subjects.

[2] In this paper, changes in volume are given in cubic millimeters per 5 cc. of part.

ton. The necessary changes to adapt the apparatus to the purposes of the present investigation were limited to: 1, the use of a prism and a mirror to shorten the over-all length of the light beam and yet retain a desirable degree of sensitivity with minimum movement of the membrane, and 2, the use of a simple dampening device, a heavy cube of rubber four inches square attached to the bracket supporting the manometer, to decrease the vibrations occasioned by the motor driving the camera which is part of the assembly. The mirror used to reflect light from the membrane of the manometer had a focal distance of six feet. By reflecting the light twice, first 180° by a prism and second, another

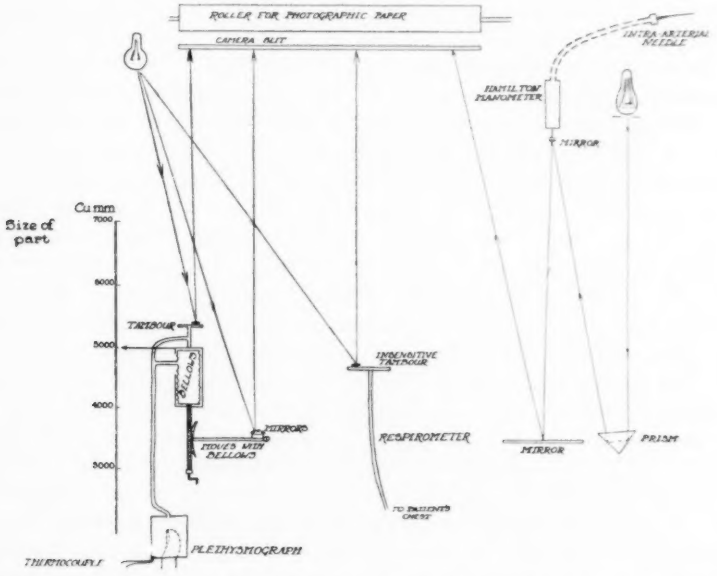

Fig. 1. Schematic drawing is reproduced of the arrangement of the various parts of the apparatus. The bellows and tambour of the plethysmograph are moved proportionately nearer the camera when records are being made from large finger tips and farther away when small finger tips are used. The angles of reflection of light are markedly exaggerated.

180° from the mirror of the membrane, it was possible to keep the total length of the apparatus within four feet (fig. 1). The Hamilton manometer and its base line mirror were mounted on a swivel stand which was firmly screwed to the baseboard of the apparatus.

The fluid used in the eighteen-inch flexible lead tube connecting the intra-arterial needle with the manometer was physiological saline solution to which were added merthiolate to a concentration of 0.01 per cent and sodium citrate to a concentration of 1 per cent. The fluid remained sterile both on aerobic and anaerobic culture. As an additional precaution against infection, the tubing was thoroughly flushed with a fresh supply of this fluid just preceding each test.

The plethysmograph of Turner was modified slightly to allow a simpler method of comparison among records of the subjects. Instead of a fixed distance between the tambour of the plethysmographic system and the camera, the system was movable on a sliding mount. The distance between tambour and camera was lengthened and shortened depending upon whether the finger tip was respectively smaller or larger than the average volume of 5 cc. The volume of the finger tip was ascertained for each subject and the apparatus adjusted accordingly. Once a tambour of desirable sensitivity was secured, the records obtained with its use were comparable for all subjects; no further calculations were necessary.

The subjects used for this investigation included four normal young adults, four adults with hypertension, and two convalescent patients free from circulatory disease. They varied in age from 20 to 40 years. Two of the normal subjects were female, all of the others were male. An attempt was made to acquaint all of these volunteers with the nature of the problem and the technique employed, by making a series of preliminary records of the volume changes of the finger tip during the week or two preceding the simultaneous recording of intra-arterial blood pressure and of fluctuations in volume of the finger tip. Previous studies (7) have shown the importance of eliminating, in so far as is possible, the distracting features of a laboratory in order to decrease the influence of extraneous stimuli upon spontaneous physiological variations. The attempt to do this during the present investigation was less than fully successful because of the necessity for having at least one observer present during each test.

The left index finger was selected for recording changes in volume and the left radial artery at the wrist for obtaining simultaneous records of blood pressure. After the subjects had been allowed to rest for a half-hour an air-tight cup was sealed to the tip of the left index finger and connected to the tambour of the plethysmograph. With only one observer, well known to the subject in the room, a record of the spontaneous changes in the volume of the finger tip (alpha waves) was made usually for a period of a half-hour. A second observer then entered the room, quickly made a small intradermal wheal with 1 per cent novocaine solution over the site of injection and inserted the needle (gauge 23) into the left radial artery. Simultaneous records were made for variable periods of time, the longest being eleven minutes and the average five minutes. Half of the subjects said that they experienced momentary pain shooting upwards towards the shoulder at the time of insertion of the needle. The pain subsided rapidly and was not present at the time the records were made.

Results. Close comparison of the alpha deflections of the finger tip with the spontaneous variations in blood pressure established the fact that they were independent of each other though their average frequencies were almost the same (about 6 times a minute). In addition, some changes in alpha deflections as large as 50 cu. mm. in the course of a minute occurred without any preceding, simultaneous or subsequent, measurable change in blood pressure. In a previous communication (3) it was reported that alpha deflections recorded simultaneously from the tips of the index fingers of opposite hands were usually but

not continuously concordant. This possibility was demonstrated again during the measurement of intra-arterial blood pressure in one subject from whom deflections were recorded of both the left and right sides. As can be seen from the illustration (fig. 2) the volumes of these varied discordantly for part of the time and neither bore any relationship to the essentially constant level of blood pressure.

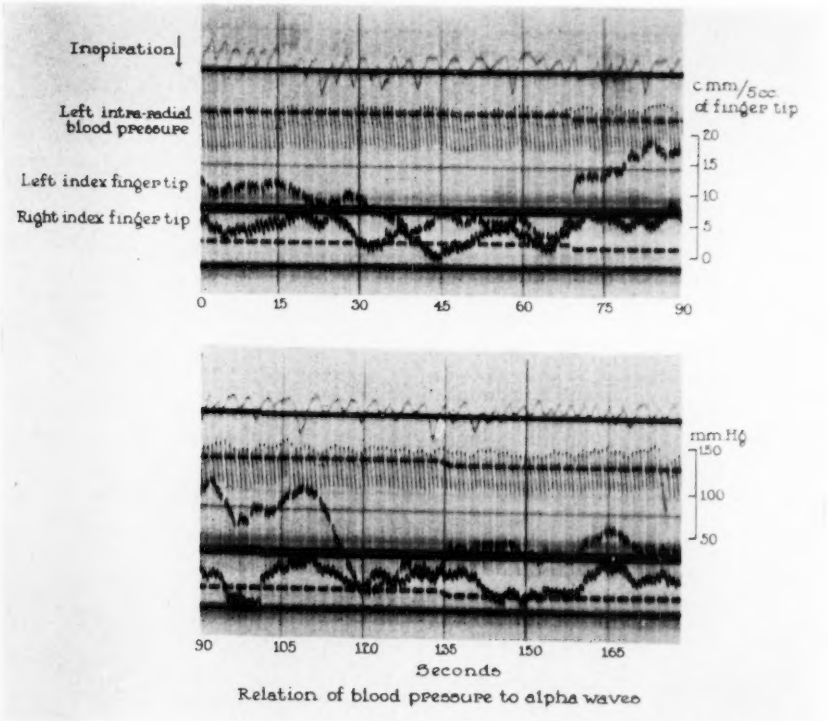

Fig. 2. A comparison between intra-arterial blood pressure and alpha deflections is shown. Alpha deflections of the two index finger tips occur independently of each other and without reference to changes in blood pressure. The record of the tip of the left index finger is the one which is interrupted by the white lines at regular intervals.

The records of intra-arterial blood pressure showed two types of rhythmic fluctuation, occurring both in systole and diastole. One occurred simultaneously with respiration. Expiration was consistently accompanied by a slight rise (2 to 5 mm. Hg). This phenomenon has recently been studied in dogs by Hamilton, Woodbury and Vogt (8) who ascribed it partly to an increase in intra-thoracic pressure and partly to an increase in cardiac output.

The other type was not so constant. Four of the ten subjects (2 normal adults, 2 hypertensive patients) showed rhythmic increase and decrease in blood pres-

sure, occurring four to six times a minute and involving a systolic change of 15 to 30 mm. Hg and a diastolic change of 10 to 20 mm. Hg (fig. 3). Although these oscillations had the frequency of Traube-Hering waves, they occurred in human subjects who were awake; classical Traube-Hering waves have been described only in morphinized or curarized animals. Steele recently has obtained similar fluctuations while using a comparable technique (4).

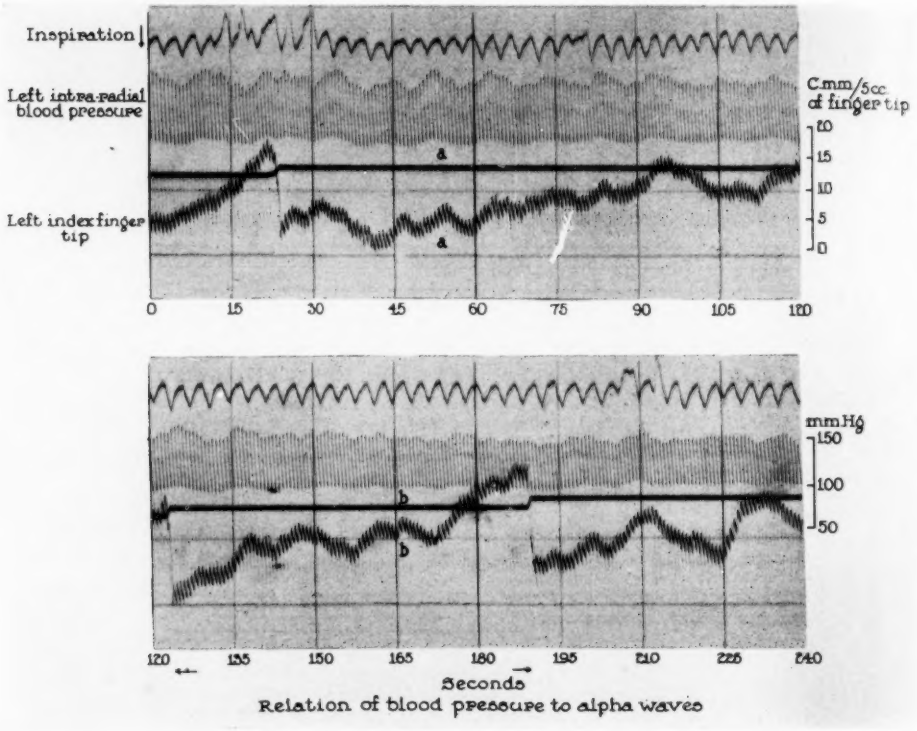

Fig. 3. Another comparison between an intra-arterial blood pressure record and alpha deflections of the index finger tip is shown. In the portion of the record marked *a*, spontaneous variations in blood pressure move in the same direction as small alpha deflections. This is an uncommon picture. The more usual occurrence is shown at *b* where alpha deflections are completely unrelated to changes in blood pressure.

Spontaneous variations in volume of the finger tip were recorded in all subjects, corresponding in frequency and size with the results previously reported (3). Before the insertion of the intra-arterial needle the size of the alpha and pulse waves was larger than after this was done. The change occurred as soon as each subject realized that the needle was about to be inserted but not at the moment of touching or piercing the skin or arterial wall although further vasoconstriction developed then. No attempt was made to measure the exact

sizes either of the alpha or pulse waves because they were obtained during a period of subjective anxiety and tension apparently related to the punctures. All the subjects admitted some anxiety at the prospect of being stuck with a needle. But this was not enough to prevent the few, in whom the introduction of the needle was not immediately successful, from volunteering a second time. A second attempt after an interval of a few days was better than continued probing. The importance of anxiety as a modifier of the size of alpha deflections corresponded with other experiences (9).

In the record of one subject a difference from the usual complete dissociation between alpha deflections and variations in blood pressure was noticed (fig. 3). During the course of approximately one minute, spontaneous decreases or increases in blood pressure (systolic and diastolic) were respectively concordant with small decreases or increases in the volume of the finger tip (fig. 3a). No other portion of this record showed similar agreement. It is significant, perhaps, that no large alpha deflections were in progress at the time. The meaning of such fleeting relationships is not known, but it would appear that a series of very small changes in volume may require to be interpreted as the effect of changes in blood pressure.

In those records illustrating a prominent respiratory influence both upon the alpha waves and upon the blood pressure, the changes occurred simultaneously. Inspiration was then associated with small decreases both in blood pressure and in the size of the finger tip. Whenever the subject momentarily held his breath, both types of response disappeared, only to return with the onset of respiration. A similar type of concordance occurred immediately following pauses in cardiac action (skipped beats). In such instances when the blood pressure fell, the direction of the alpha deflection was downward until the next heart beat occurred. Then the blood pressure was immediately restored to its original level (within one or two beats) but a delay of about 15 seconds intervened before the volume of the finger tip was completely regained. In this period of recovery, the volume of the finger tip gradually increased while the blood pressure was maintained at a constant level.

Discussion. Although it has been shown that variations in volume of the finger tip (alpha deflections) are independent of normal spontaneous changes in blood pressure, the circumstances under which this investigation was carried out actually made the demonstration of this independence difficult. The average size of alpha deflections depends in great part upon the degree of relaxation of the subject at the time the record is made (9). When anxiety and tension are prominent, alpha deflections tend to be small. Throughout the period of simultaneous recording of blood pressure and alpha waves, the latter were smaller than before the insertion of the needle into the artery. Had it been possible to obtain the records of blood pressure without the introduction of a needle, there would have been less cause for anxiety on the part of the subject. Alpha deflections would then have been larger and the lack of correlation between alpha deflections and changes in blood pressure even more impressive. It must be emphasized that the change from large alpha deflections obtainable be-

fore to smaller ones afterward did not occur at the moment of penetration into the arterial wall, but when the patient was first aware that the needle was to be inserted. This point is emphasized in order to exclude the possibility that the change in size of alpha deflections resulted from a reflex initiated by irritation of the vessel well.

The fact that alpha deflections are independent of changes in blood pressure is supported by the frequency with which records obtained simultaneously from the finger tips of opposite hands show discordant deflections. This occurs in about 25 per cent of the number of deflections (3). Similar discordance has been noticed when simultaneous records are made from the index finger and second toe. As a matter of fact, the difference in time (0.3 sec.) between constriction of a finger tip and that of a toe tip following the application of an external stimulus such as light or heat upon a distant part of the body is far greater than might be expected if such responses resulted primarily from a change in systemic blood pressure (fluid transmission) and approximates that to be expected from the transmission of nerve impulses to constricting elements in fingers and toes (10).

One of the main reasons for studying the relationship of spontaneous variations in blood pressure to the occurrence of alpha deflections was the hope that a better understanding of the mechanism underlying alpha deflections would evolve. But, in this regard, the evidence obtained was only of negative value. But upon what alpha deflections depend and what is the rôle of such changes in the economy of the body are still unsolved problems. Certainly all superficial tissues do not exhibit such fluctuations, at least not of the magnitude discovered in fingers and toes. Hertzmann and Roth have recently emphasized this point anew (11).

This difference in behavior in different parts of the body is not limited to variations in volume, but has also been noticed in the loss of water from the skin. This, like the activity of blood vessels, is under the control of the sympathetic nervous system. The rate of water loss from fingers and toes, for example, is greatly in excess of that from forearm or chest during rest in a comfortable evnironment and undergoes spontaneous variations which are as impressive as are changes in volume (12, and unpublished data). An inquiry into the nature of these phenomena is not answered by making this comparison but it suggests that alpha deflections are not isolated physiological oddities.

Chambers (13) has observed alternating constriction and dilatation of individual arterioles. The observations were made in rats, but it is a near step toward regarding alpha deflections in human fingers as comparable phenomena. If the constriction and dilatation of arterioles are mechanisms which provide for the exchange of tissue fluids, then the study of alpha deflections assumes a new importance in understanding the transport of fluids in the body.

SUMMARY

By the simultaneous use of a plethysmograph for recording changes in volume of the tip of the left index finger and of an intra-arterial manometer for obtaining

synchronous readings of the blood pressure of the left radial artery, it was shown that the spontaneous variations (increase or decrease) in volume of the finger tip are not concordant with spontaneous changes in blood pressure (? Traube-Hering waves) and are present even in the absence of measurable variations in blood pressure. A few exceptions were noticed. Rises in systemic blood pressure during expiration were accompanied by variable but small increases in volume of the finger tip. Marked lowering of blood pressure accompanying cardiac asystole was reflected in a decrease in volume. The rule then seems to be that variations in the volume of the finger tip usually go on independently of changes or lack of change in blood pressure though under certain conditions there may be a transitory relationship. When present, it is manifested by an increase in volume when there is a rise in blood pressure.

REFERENCES

(1) Burton, A. C. This Journal **127:** 437, 1939.
(2) Hertzmann, A. B. and J. B. Dillon. This Journal **127:** 671, 1939.
(3) Burch, G. E., A. E. Cohn and C. Neumann. This Journal **136:** 433, 1942.
(4) Steele, J. M. J. Mt. Sinai Hosp. **8:** 1042, 1942.
(5) Turner, R. H. J. Clin. Investigation **16: 777**, 1937.
(6) Hamilton, W. F., G. Brewer and I. Brotman. This Journal **107:** 427, 1934.
(7) Neumann, C., A. E. Cohn and G. E. Burch. J. Clin. Investigation **21:** 651, 1942.
(8) Hamilton, W. F., R. E. Woodbury and E. Vogt. This Journal **125:** 130, 1939.
(9) Neumann, C., W. T. Lhamon and A. E. Cohn. To be published.
(10) Burch, G. E., A. E. Cohn and C. Neumann. J. Clin. Investigation **21:** 655, 1942.
(11) Hertzmann, A. B. and L. W. Roth. This Journal **136:** 692, 1942.
(12) Burch, G. E., A. E. Cohn and C. Neumann. Am. Heart J. **23:** 185, 1942.
(13) Chambers, R. Personal communication.

VARIABILITY OF CERTAIN FACTORS IN THE BLOOD PICTURE OF WOMEN[1,2]

EVA G. DONELSON, JANE M. LEICHSENRING AND MARGARET A. OHLSON

From the Division of Home Economics, University Farm, St. Paul, Minnesota, and Division of Home Economics, Iowa State College, Ames

Received for publication October 19, 1942

The usual criterion for determining the severity of an anemia is the extent of the divergence of the individual's blood picture from a range of values which have been established from measurements on many persons of the same sex and age. This range represents the difference between individuals and gives no indication of the variability that may be expected in the same person who is the subject of repeated observations. The latter type of information would be of value in interpreting clinical findings on subjects whose progress toward recovery is being followed by successive measurements. Studies reported in the literature dealing with the intra-individual variability have included relatively small numbers of subjects and few blood factors.

Platt and Freeman (4) made monthly hemoglobin determinations over a period of six months on 29 children ranging in age from 21 to 44 months and noted a maximum difference in the mean values of 2.9 grams per 100 ml. of blood in that interval of time. Weekly determinations of the hemoglobin values of 30 young medical students, men and women, were made by Ingersoll (1) for a period extending from October to January. The difference between maximum and minimum values varied for the different subjects from 0.4 to 2.7 grams per 100 ml. of blood, with the women showing greater differences than the men. A further contribution to this field is the study by Jellinek (2) who investigated the intra-individual variability in erythrocytes and leucocytes in 30 normal men, as well as a group of schizophrenics. He found a significant increase in the intra-individual variance in both erythrocytes and leucocytes with increasing time-intervals between observations. He noted further that the individual is significantly more homogeneous than the group with respect to both these factors.

The present study reports the results secured on a large number of healthy college women on whom observations were made at intervals varying from 1 week to 6 months. Two or more observations were made on each subject. In addition, the results of a series of day-to-day determinations on four women over periods of from 27 to 39 days are recorded.

Procedure. In most cases blood samples were secured by finger-tip puncture

[1] Approved for publication by the Advisory Committee as paper no. 18 of the Regional Project of the North Central States Relating to the Nutritional Status of College Women.

[2] Paper 2035 Scientific Journal Series, Minnesota Agricultural Experiment Station. Journal Paper No. J1051 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 538.

between 8 and 9 a.m. from subjects in basal condition.[3] Hemoglobin values were determined on duplicate samples by the Newcomer method using a disk which had been standardized by the oxygen-capacity method. Red and white cell counts were each made on two separate dilutions of blood using Thoma automatic pipettes, certified by the United States Bureau of Standards. Packed cell volume was measured by means of Van Allen hematocrit tubes, with heparin as the anti-coagulant. Duplicate samples were centrifuged for 30 minutes at

TABLE 1

Intra-individual variations in the blood picture of women, based on periodic measurements

TIME INTERVAL	NUMBER OF CASES	MEAN OF INITIAL READINGS	MEAN OF FINAL READINGS	MEAN DIFFERENCE	STANDARD ERROR OF A DIFFERENCE
Erythrocytes (millions per c.mm.)					
Within one week	227	4.25	4.25	0.00	0.20
Within two to six weeks	111	4.25	4.23	−0.02	0.29
Within seven weeks to six months	53	4.40	4.52	0.12	0.30
Hemoglobin (grams per 100 ml.)					
Within one week	238	13.10	13.05	−0.05	0.79
Within two to six weeks	137	12.96	12.89	−0.07	0.87
Within seven weeks to six months	92	13.57	13.68	0.11	1.20
Packed cell volume (per cent)					
Within one week	229	39.30	39.06	−0.24	1.63
Within two to six weeks	112	38.93	38.76	−0.17	1.72
Within seven weeks to six months	51	39.14	39.51	0.37	2.35
Leucocytes (thousands per c.mm.)					
Within one week	217	6.16	6.09	−0.07	1.26
Within two to six weeks	93	6.00	5.99	−0.01	1.72
Within seven weeks to six months	33	6.16	6.05	−0.11	1.53
Erythrocyte diameter (microns)					
Within one week	68	7.38	7.36	−0.02	0.11
Within two to six weeks	36	7.28	7.26	−0.02	0.15
Within seven weeks to six months	37	7.22	7.34	0.12	0.20

2750 revolutions per minute. For red cell diameter measurements, dilutions were prepared with Hayem's solution, one diameter of 200 round cells being measured immediately by means of a calibrated filar micrometer.

RESULTS AND DISCUSSION. In table 1 is shown the intra-individual variation in the blood picture of healthy young women which occurs when observations are made within one week, within two to six weeks, and within seven weeks to six months. Scrutiny of the table reveals that the mean difference between the

[3] One hundred of the samples were secured between 8 a.m. and 12 noon following a light breakfast.

initial and final values for the various factors is very small. The standard error of a difference, which is a measure of variability and defines the limits above and below the mean within which approximately two-thirds of the cases may be expected to fall, increases sharply with extension of the time-interval between the determinations. This observation is in agreement with the previously cited finding of Jellinek.

As an indication of the variation that may be anticipated in an individual blood picture during a limited period of time, a more intensive study on four individuals was made. The means and standard errors of the day-to-day values were computed and are given in table 2. In a normal individual the variation in blood values is influenced not only by true physiological alterations but also by the inaccuracies that are inherent in the methods employed. Since the measurements in the present study were made by skilled workers, the changes noted are such as may be expected clinically in consecutive observations of these blood factors on the same subject. It will be noted that the variability in the

TABLE 2

Intra-individual variations in the blood picture of women, based on day-to-day measurements

SUBJECT	DAYS OF OBSERVATION	ERYTHROCYTES		HEMOGLOBIN		PACKED CELL VOLUME		ERYTHROCYTE DIAMETER	
		Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error
		millions/ c.mm.	*millions/ c.mm.*	*grams/100 ml.*	*grams/100 ml.*	*per cent*	*per cent*	*microns*	*microns*
1	27	4.57	0.10	13.94	0.81	38.04	1.76	7.18	0.18
2	28	4.49	0.18	13.63	0.54	39.59	1.62	7.13	0.10
3	39	4.00	0.14	11.53	0.69	36.61	2.00		
4	39	3.94	0.14	12.13	0.71	38.20	1.26		

blood data of these four subjects was no greater than might have been expected when the variance obtained for the larger group of subjects in the two to six weeks time-interval is used as a criterion (table 1). In those few instances in which the data were somewhat more variable the differences were not great.

A number of workers have investigated the effect of menstruation on the blood picture of women (Smith and McDowell, 7; Reich and Green, 5; Smith, 6; and Leverton and Roberts, 3). These workers report that fluctuations occurred in the blood factors studied during the menstrual cycle irrespective of the phase of the cycle in which the observations were made. Although Ingersoll (1) reported that the women in her study manifested a greater intra-individual variability in hemoglobin than did the men, a comparison of the variances in erythrocyte and leucocyte counts within one week for the women subjects in the current study, 0.20 million and 1.26 thousands, respectively, with those reported by Jellinek for 30 normal men, 0.27 million and 1.76 thousands, respectively, shows a somewhat lesser variability for the women than for the men. However, due to the wide difference in the number of cases studied no conclusion can be drawn as to the comparative variability of the two sexes.

SUMMARY

Erythrocyte and leucocyte counts, hemoglobin and packed cell volume determinations, and erythrocyte diameter measurements were made on a large group of healthy young women at intervals varying from one week to six months. The standard error of a difference between the initial and final value was computed for each factor for three time-intervals, i.e., within one week, within two to six weeks, and within seven weeks to six months. These showed that the intra-individual variability increased with extension of time between measurements.

Day-to-day determinations on four subjects for periods ranging from 27 to 39 days indicated that the variability in the blood factors for these subjects was in agreement with that found for the larger group.

REFERENCES

(1) Ingersoll, W. J. Lab. and Clin. Med. **21:** 787, 1936.
(2) Jellinek, E. M. Human Biology **8:** 581, 1936.
(3) Leverton, R. M. and L. J. Roberts. J. A. M. A. **106:** 1459, 1936.
(4) Platt, V. E. and R. G. Freeman, Jr. Proc. Soc. Exper. Biol. and Med. **27:** 687, 1930.
(5) Reich, C. and D. Green. Arch. Int. Med. **49:** 534, 1932.
(6) Smith, C. This Journal **114:** 452, 1936.
(7) Smith, C. and A. M. McDowell. Arch. Int. Med. **43:** 68, 1929.

ROENTGENKYMOGRAPHIC DETERMINATION OF CARDIAC OUTPUT IN SYNCOPE INDUCED BY GRAVITY[1]

H. S. MAYERSON

From the Laboratory of Physiology, Tulane University of Louisiana, New Orleans

Received for publication October 23, 1942

A common type of syncope is that which occurs after prolonged, quiet standing. Some individuals can maintain the upright posture for relatively long periods of time, whereas others show a tendency to faint soon after assuming the position. A subject may tolerate relatively long periods of quiet standing on certain occasions but not on others. Collapse, when it occurs, is assumed to be due to cerebral anemia consequent to a diminished venous return, resulting from excessive pooling in the capillaries and veins of the sub-cardiac tissues. The quantitative demonstration of the decrease in cardiac output under these conditions has been difficult. Thus in a previous investigation (1) in which the acetylene method was used, we were unable to find any greater decrease in cardiac output in fainters on prolonged standing than in non-fainters. However, the time required for the measurement of the cardiac output by this method and the necessity of obtaining the full co-operation of the subject precluded its use in the period just before collapse occurred. This was particularly true in those cases in which the onset of syncope was precipitate and rapid (see also 2, 3).

Two methods for the calculation of cardiac output which have been recently introduced seem better adapted to the problem: the ballistographic method of Starr and Rawson (4) and the roentgenkymographic method of Keys and his associates (5). Both are rapid and require a minimum of co-operation on the part of the subject. The vertical ballistocardiograph has been used by Starr and Rawson for studying the changes in cardiac output on arising. These investigators were unable, however, to use individuals subject to fainting because of uncontrollable muscular movements which ruined the record long before any symptoms set in. They failed to find a decrease in the cardiac output in six experiments in which there were only transient symptoms of faintness, lightheadedness or dizziness. On the contrary, the symptoms were often experienced during a period in which the cardiac output was definitely greater than that determined when they were absent. They state that a slight diminution of blood pressure was usually, but not always, observed concomitant with these symptoms; but in no case was the remaining pressure insufficient to raise blood to the top of the head.

We have used the roentgenkymographic method to calculate the standing cardiac outputs of eight adult males between the ages of twenty-one and forty. Three of these (H. S. M.; W. J. T., Jr.; W. D. D., Jr.) are non-fainters who can stand passively for at least twenty minutes with no manifestations of cardiovas-

[1] Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

cular embarrassment. One, H. L. B., perspires freely, becomes pale and lightheaded, but does not faint. The remaining four subjects have consistently shown signs of oncoming syncope within this period. The latter group includes one of the quarterbacks on the football team (T. G.), a former varsity football man (L. A. T.), and a former member of the boxing team (P. F. C.). Two of the subjects—H. S. M., a non-fainter, and L. A. T., a fainter—had served in the previously mentioned study in which the cardiac output alterations with posture had been determined by the acetylene method (1).

Roentgenkymograms were taken in mid-respiration with the subjects standing, using a target-film distance of 30 inches, exposure factors of 100 ma and 100 kv, and an exposure time of 1.5 seconds. The first kymogram was taken within three or four minutes after the subject was placed in position and was followed, in most instances, by a second exposure at the end of twenty minutes of quiet standing. When syncope seemed imminent before this time, an attempt was made to take the kymogram just before complete collapse. In one experiment (table 1, expt. 12), four films were taken at five minute intervals during the standing period. The diastolic and systolic volumes and the stroke output were calculated according to method B of Keys and collaborators.

Since our chief interest was in comparing the cardiac outputs at the beginning and at the end of the standing period, particular care was exercised in treating each pair of kymograms in the identical manner. Our use of the short focal distance (30 in.) introduced errors of distortion and magnification which cannot be adequately corrected. These were minimized, however, by use of the formula:

$$\left(\frac{D - X}{D}\right)^2 \cdot \text{Area (measured)} = \text{Area (corrected)},$$

D being the target-film distance (30 in.) and X, the heart-film distance. The latter value was taken as $\frac{1}{3}$ of the anterior-posterior chest diameter as measured with a pelvimeter, plus the distance from the outside of the kymograph panel to the film. In spite of the approximations inherent in our method, the values for cardiac output obtained agree rather closely with those previously determined by the acetylene method under similar conditions on the two subjects mentioned above. It should be emphasized, however, that the values given below are to be considered as having relative rather than absolute accuracy.

The results of our experiments are given in table 1. The relatively high values for stroke outputs are due to the fact that the kymograms were not taken under basal conditions and the majority of the subjects were not trained for the procedure. The lowest initial values in the series are those of the two veteran subjects, H. S. M. and L. A. T. The three non-fainters show only small changes in heart and stroke volume and in cardiac output. These may be considered to be within the limits of error of the method. The alterations in cardiac output vary with those in pulse rate. In two experiments the increase in the latter function results in a rise in the cardiac output; in the remaining three experiments the percentage decrease in cardiac output is greater than that in stroke output due to the diminished pulse rate at the end of the standing period. Our findings are

thus in general agreement with those of Starr and Rawson (4), who reported that the values for cardiac output in non-fainting subjects reach a plateau after a minute of standing, a level which is maintained with insignificant variations for at least ten minutes.

TABLE 1

SUBJECT	SURFACE AREA	EXPT. NO.	INTERVAL BETWEEEN FILMS	HEART VOL.		ΔV	STROKE OUTPUT (1.44ΔV)	PULSE RATE	CARDIAC OUTPUT	P. C. CHANGE		REMARKS
				Diast.	Syst.					Stroke output	Cardiac output	
Non-fainters												
	m^2		*min.*	*cc.*	*cc.*		*cc.*	*min.*	*l./min.*			
H. S. M.	1.87	2-1		440.5	415.7	24.8	35.7	90	3.21			No circulatory embarrassment
		2-2	20	454.0	431.5	22.5	32.4	86	2.79	−9.3	−13.0	
		11-1		436.7	406.0	30.7	44.2	88	3.89			No circulatory embarrassment
		11-2	20	436.5	405.5	31.0	44.7	95	4.24	+1.0	+8.2	
W. J. T., Jr.	1.93	4-1		459.0	429.0	30.0	43.2	104	4.49			No circulatory embarrassment
		4-2	20	462.2	451.6	30.6	44.1	100	4.41	+2.1	−1.8	
		9-1		389.0	356.0	33.0	47.5	104	4.94			No circulatory embarrassment
		9-2	20	385.5	353.4	32.1	46.2	96	4.43	−2.7	−10.3	
W. D. D., Jr.	1.89	13-1		476.0	424.0	52.0	74.9	86	6.44			No circulatory embarrassment
		13-2	20	462.5	414.3	48.2	69.4	96	6.66	−7.3	+3.2	
Intermediate												
H. L. B.	1.95	10-1		564.0	504.5	59.5	85.7	85	7.28			Dizzy, lightheaded, *etc.* No fainting
		10-2	20	576.0	531.0	45.0	64.8	92	5.96	−24.4	−18.1	
Fainters												
T. G.	2.10	5-1		435.0	385.0	50.0	72.0	103	7.42			On verge of syncope
		5-2	18	417.0	379.3	37.7	54.2	104	5.64	−24.7	−23.9	
J. A. G., Jr.	1.67	6-1		402.0	354.7	47.3	68.1	106	7.22			On verge of syncope
		6-2	20	354.7	323.8	30.9	44.5	125	5.56	−34.7	−23.0	
L.A.T.	1.93	3-1		523.5	481.5	42.0	60.5	61	3.69			
		3-2	19	530.7	502.5	28.2	40.6	69	2.80	−33.0	−24.1	Fainted later
		8-1		497.4	461.3	36.1	52.0	68	3.54			Sweating, lightheaded, dizzy.
		8-2	27	490.0	456.0	34.0	48.9	68	3.33	−6.0	−6.0	
		12-1		523.5	481.0	42.5	61.2	75	4.59			Considerable movement of arms
		12-2	5	495.6	468.0	27.6	39.7	66	2.62	−35.1	−43.0	
		12-3	10	523.5	490.0	33.5	48.2	75	3.62	−21.2	−21.1	On verge of syncope
		12-4	15	523.5	490.0	33.5	48.2	66	3.18	−21.2	−30.8	
P. F. C.	1.41	1-1		486.4	449.0	37.4	53.9	86	4.64			
		1-2	16	483.0	428.3	54.7	78.8	86	6.78	+46.2	+46.2	Fainted (see text)
		7-1		457.5	426.1	31.4	45.2	89	4.02			Considerable twitching, *etc.*
		7-2	20	462.0	420.7	41.3	59.5	95	5.65	+31.6	+40.5	

The findings for H. L. B., who was classified as an intermediate, can be correlated rather closely with his subjective manifestations. His stroke output decreased more than that of the non-fainters but less than that of the fainters. This drop in stroke output is minimized somewhat by the concomitant rise in the

pulse rate. With one exception to be discussed below, all of the fainting subjects show significant and marked decreases in their stroke and cardiac outputs. The changes in experiment 6 are of especial interest. After ten minutes of quiet standing, the subject was sweating profusely and was slightly dizzy. Signs and symptoms of syncope became increasingly manifest and the subject was urged to make every effort to remain in position for the twenty-minute test period. He was on the verge of collapse when the second kymogram was taken. Analysis of the kymograms indicates a decrease in diastolic and systolic volume of 11.7 and 7.1 per cent respectively and a marked drop in stroke volume. These changes are unquestionably correlated with the marked tachycardia evident at the end of the standing period. The increase in the pulse rate, accompanied by a diminished venous return, does not allow sufficient time for the adequate filling of the auricle. The ventricular volume becomes smaller and the stroke output is further decreased to such an extent that, in spite of the increased rate, the cardiac output remains low.

Experiment 12 on L. A. T. indicates that the stroke and cardiac outputs in fainters decrease considerably within a few minutes after the standing position is assumed, before any subjective signs or symptoms are evident. Thus the subject in this experiment reported feeling lightheaded and dizzy only after the third film was taken—about thirteen minutes after he assumed the standing position, and syncope was not imminent until five minutes later. Similar observations were made on two fainters examined under the fluoroscope. The changes in heart size were of sufficient magnitude to enable the observer to predict whether or not the subject would faint several minutes later.

The secondary rise in the stroke and cardiac outputs in experiment 12, as seen in the last two observations, is probably due to the restlessness evident when the standing period is prolonged beyond ten minutes, particularly in fainters who exhibit considerable swaying and involuntary movement of the arms after standing for seven to ten minutes. The importance of these muscular movements in increasing the stroke output even to the extent of preventing syncope is illustrated by experiment 8 on this same subject. This individual is our most consistent fainter, who, in experiments extending over four years, has seldom been able to maintain the position of quiet standing for twenty minutes without acute embarrassment. On this occasion he was perspiring freely and complained of being light-headed and dizzy at the end of eighteen minutes. He became restless, swayed and moved his arms considerably. Soon after, he reported feeling much better; and the second film, taken at the end of twenty-five minutes, showed a much smaller change in cardiac output than in other experiments.

Even more striking in this connection are the experiments on P. F. C. In the first experiment (expt. 1), the initial film was taken after three minutes of standing. Ten minutes later he was dyspneic, pale and showed fine fibrillary twitchings of the neck and arm muscles. He was sweating profusely and complained of a dry mouth. Two minutes after this time, the twitchings were much worse and he reported feeling weak and almost out. Five seconds later, before the second film could be taken, he fainted and would have fallen to the floor had he

not been securely strapped to the frame of the apparatus. He was twitching violently and exhibited a series of tonic and clonic convulsions. The period of unconsciousness lasted approximately a second, following which the muscular contractions subsided. The second film was taken at this time. The cardiac output calculated from it was 46.2 per cent higher than at the beginning of the standing. Similar results were obtained in a second experiment (expt. 7) on the same subject, who succeeded this time, however, in standing for twenty-three minutes without actually fainting.

These results strengthen the conviction based on previous experiments (6) that absence of adequate muscular contraction is the primary factor leading to a secondary failure of the circulation when standing is prolonged. During the period preceding collapse, decreased tissue temperatures, pallor, tachycardia and sweating, which are invariably present, attest the fact that the sympathetic centers are being stimulated rather than depressed. When an individual changes from the lying to the standing position there is a reduction in the distention of the aortic and sinus walls which evokes reflex compensatory responses such as a speeding of the pulse rate and a general vasoconstriction. The latter adjustment diminishes the volume flow and prevents a flooding of the capillary reservoirs in the subcardiac regions. Such a compensatory mechanism is only partially effective, however, for once the blood succeeds in getting through to the veins, it has difficulty in returning to the heart. In this way a vicious cycle is created. The volume flow is reduced not only as a result of the difficulty of returning blood to the heart against gravity but also by the compensating mechanism itself. If muscle tonus is low, there will therefore be a greater tendency for stagnation to occur and for the venous flow to be decreased to such an extent as to fail to meet the demands of gas transport for the individual at that particular time. The twitchings and muscular movements (sometimes convulsions) which often precede syncope are thus an attempt at compensation for the lessened venous return.

The usual classification of postural syncope as "vasovagal" is unfortunate, for cardiac inhibition is seldom seen until just before or during the syncope. Since the collapse occurs because of a diminished venous return it is obvious that changes in pulse rate would be contributory rather than causative factors. Marked changes in either direction would result in a decreased cardiac output. Thus the occurrence of syncope in experiments 3 and 12 on subject L. A. T. may be due to the presence of strong vagal tone preventing the usual compensatory increase in pulse rate. On the other hand, experiment 6 indicates that syncope may occur in the presence of tachycardia and suggests that the increase in the pulse rate may be great enough to shorten the time for diastolic filling appreciably so that, in the presence of a diminished venous return, the stroke output is markedly decreased. The cardiac output may thus fall because of the increased pulse rate.

As we have previously indicated, three of our fainting subjects were athletes in good physical condition. One of them, T. G., was playing football during the period he was serving as subject for these experiments. Syncope occurred in

these individuals at relatively high levels of cardiac output. Starr and Rawson (4) concluded that "there is a limit below which the cardiac output of a standing subject cannot go with safety, and that this limit is located near the middle of the normal range found in recumbent subjects, whose circulations are governed by different and less rigid requirements." Since the resting cardiac output of the athlete is usually higher than that of the non-athlete (5) it seems likely that the standing requirements of the former are higher than those of the latter. Athletes might thus be expected to show signs and symptoms of circulatory embarrassment at levels of cardiac output actually higher than those present in non-fainters under similar conditions. Under conditions in which the pumping and massaging actions of muscular contraction were at a minimum, athletes would be more severely handicapped than those individuals whose standing requirements are less and whose margin of safety in the standing position is therefore greater.

SUMMARY

Individuals who can stand quietly for at least twenty minutes show insignificant changes in the stroke and cardiac outputs during the standing period as determined by the roentgenkymographic method. Fainters show a marked decrease in these functions under the same conditions. If no marked movement occurs, the stroke output just before syncope, is 25 to 35 per cent less than at the beginning of the standing period, while the cardiac output has diminished 21 to 43 per cent. The development of syncope in quiet standing is primarily due to the absence of adequate muscular contraction which results in a diminished venous return. The vasomotor failure is secondary.

Acknowledgments. I am grateful to Drs. J. N. Ané and A. Mayoral of the Department of Radiology, School of Medicine, Tulane University for placing the necessary equipment at my disposal and for assisting in the experiments. My thanks are also due Dr. H. M. Friedell of the University of California Hospital and Dr. H. E. Ungerleider of the Medical Department of the Equitable Life Assurance Society for suggestions concerning the treatment of the data.

REFERENCES

(1) Sweeney, H. M. and H. S. Mayerson. This Journal **120:** 329, 1937.
(2) Schellong, F. and M. Heinemeier. Ztschr. f. d. ges. exper. Med. **89:** 61, 1933.
(3) Schneider, E. D. and C. B. Crampton. This Journal **110:** 14, 1934.
(4) Starr, I. and A. J. Rawson. This Journal **134:** 403, 1941.
(5) Keys, A., H. L. Friedell, L. H. Garland, M. F. Madrazo and L. G. Rigler. Am. J. Roentgen. and Rad. Therapy **44:** 805, 1941.
(6) Mayerson, H. S. and G. E. Burch. This Journal **128:** 258, 1940.

DISAPPEARANCE CURVES OF THE DYE T-1824 AFTER ITS INJECTION INTO THE BLOOD STREAM[1]

BARRY G. KING, KENNETH S. COLE AND ENID T. OPPENHEIMER

From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York

Received for publication August 22, 1942

Literature on the determination of blood volume by the dye method reveals a conspicuous lack of agreement as to the interpretation of the dye curve (dye concentration vs. time after injection), and its utilization for calculating plasma volume.

The initial part of the time concentration curve is the resultant of both mixing of the dye with the blood and dye loss during this period. Direct quantitative determination of dye loss cannot be made during the period required for uniform distribution in the circulation. If the dye loss during this period is appreciable, it is evident that this must be allowed for in the calculation of plasma volume. As Erlanger (1921) pointed out, any determination of plasma volume by the dilution method is dependent upon knowledge of the exact rate at which the substance leaves the circulation.

The difficulty of deciding which particular straight line should be drawn through the time-concentration curve to represent average rate of dye disappearance during the distribution period led us to believe that further information on the dye curve was needed. The object of our investigation, therefore, was to obtain a more detailed knowledge of the time-concentration curve at every period, beginning immediately after injection and continuing for many hours thereafter.

PROCEDURE AND RESULTS. Some fifty experiments were performed on normal dogs, using the blue dye T-1824, as described by Gregersen and Stewart (1939). The dye was injected into a jugular vein, and a blood sample was drawn from the contralateral vein, sometimes only 30 seconds after injection. During the early stages samples were drawn frequently, often at half-minute intervals, but at longer intervals during the later stages. Dye concentrations of the samples were determined by spectrophotometric measurements of the serum.

Figure 1A gives examples of typical curves from experiments lasting about two hours, obtained by plotting the dye density of each successive sample as ordinate and the time after injection as abscissa. The form of the curves is in general similar to concentration curves for other of the more slowly disappearing dyes reported by previous observers (Erlanger, 1921; Smith, 1925; Robinow and Hamilton, 1940; and others). After the characteristic initial rise, the concentrations of successive samples decrease at a continually diminishing rate. Figure 1B shows that the same type of curve is maintained even when the observations

[1] Preliminary report: Federation Proceedings, Vol. 1, March 1942.

are continued for as long as 26 hours, the dye density continually decreasing during this period, but always at a progressively diminishing rate.

Discussion. Plasma volume is customarily calculated either from the dye density of a single blood sample or from the relation of successive samples. When the investigator relies on a single sample, the decision as to the moment of sampling is of great importance. The blood must be drawn before significant dye loss has occurred, yet it is clear from the curves that the concentration changes most rapidly during the first ten minutes after injection, so that this period offers a maximum opportunity for error.

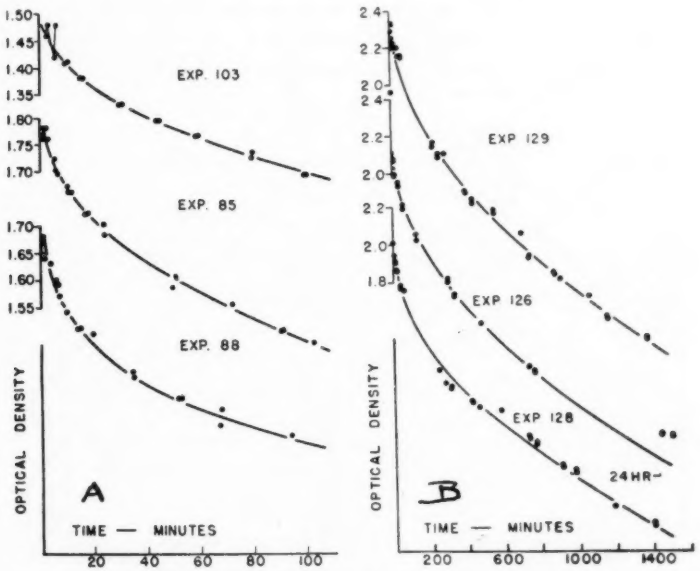

Fig. 1. Concentration curves of T-1824. Ordinates—optical density; abscissae—time, linear scale.

A, experiments lasting 100 minutes; B, experiments lasting 24 hours.

An alternative method, first suggested by Erlanger (1921), has been adopted by many observers (Sunderman and Austin, 1936; Gibson and Evans, 1937; Kennedy and Millikan, 1938; Gregersen and Stewart, 1939; Gregersen, 1941) in an endeavor to allow for dye disappearance during the mixing period. If, after mixing is complete, a curve can be established as representing dye loss from the blood stream, then backward extrapolation of this curve to the time of injection may be held to give the theoretical dye density at this instant. This is warranted only if the extrapolated line constitutes a true expression of the dye disappearance from the moment of injection.

It appears reasonable to assume that the rate of loss of dye injected in moder-

ate amounts would be proportional to the concentration, as is characteristic of many biological processes. This leads to the equation

$$c = c_o \cdot e^{-t/T} \quad \ldots\ldots (1)$$

where c_o is the concentration at the time of injection, c is the concentration at time t after injection, and T is a time constant. However, points obtained by plotting t and log c for data such as in figures 1 and 3 do not fall on a straight line, and equation (1) does not hold over any extended period, although a sufficiently short segment of these curves will always appear to be a straight line, particularly where the space allotted to the variables on the co-ordinates is dis-

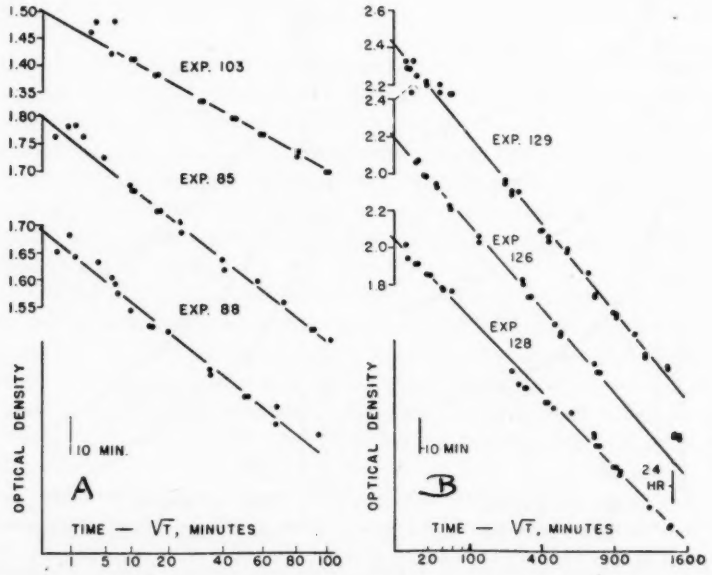

Fig. 2. Concentration curves of T-1824. Ordinates—optical density; abscissae—time, square root scale.

A, experiments lasting 100 minutes; B experiments lasting 24 hours.

proportionate. Dye concentration curves as often plotted are flattened as a result of emphasis on the time coordinate at the expense of the concentration coordinate. As a consequence, previous investigators have interpreted the selected portion of the curve as approximating a straight line given by

$$c = c_o(1 - t/T) \quad \ldots\ldots (2)$$

where the symbols are as in (1). Figure 3 shows examples of this approximation. Its use for the determination of the initial concentration will be called the "linear extrapolation method," and will be designated by L.E.M. in subsequent discussion. The use of the L.E.M. for the calculation of plasma volume has already been criticized by Robinow and Hamilton (1940).

It may be seen from figure 3, curves I (Kennedy and Millikan, 1938), II (Gibson and Evans, 1937) and IV, that, if early points are included in the observations, they do not lie on the superimposed straight line. While it is evident that the mixing portion of a dye concentration curve must necessarily deviate from the true representation of dye loss during the distribution period, deviations from extrapolations of linear functions do not constitute an adequate criterion of mixing. Early points of a linear time-concentration curve always fail to fall on an imposed straight line, whether the period of observation is 12 minutes or 24 hours. This may not be immediately apparent from inspection of individual curves covering a limited time range, but will show up conclusively if the period of observation included in the curve is extended. This is illustrated also by figure 3; curves I and II were redrawn from the literature without modification, while curve IV was taken from one of our own experiments. The three straight

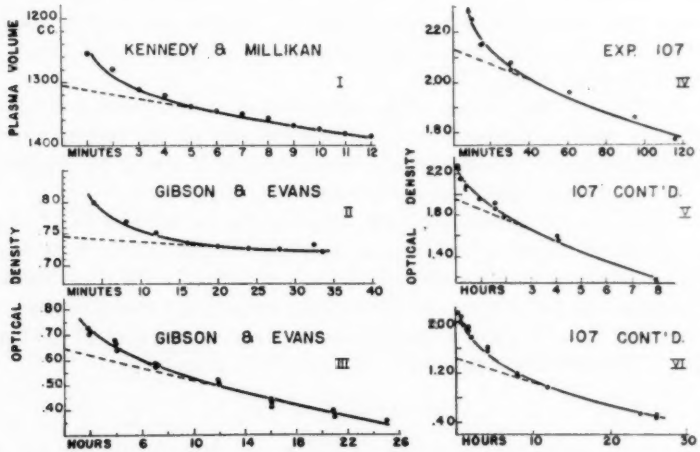

Fig. 3. Time-concentration curves plotted for varying periods after dye injection.

lines in curve III (Gibson and Evans, 1937), and in curves V and VI have been drawn in by us to show the deviation of points at the beginning of the curves. Such deviations, occurring several hours after injection of the dye, obviously cannot be attributed to incomplete mixing.

Since any limited portion of such a curve will serve equally well as approximating a straight line, the portion utilized has varied with the views of the investigator. In this approximation of dye loss as a linear function of time, the choice of the particular line of best fit must be revised whenever the time range is extended. This is illustrated in figure 3 by curves IV, V and VI from a single experiment, in which the interval after injection has been extended for each successive graph; the resulting extrapolated lines cross the ordinate axis at successively lower levels.

Since dye loss cannot be represented by a single linear function, the plasma volume calculated by the L.E.M. will vary according to the portion of the curve

from which the extrapolation is made. The earlier the period chosen, the steeper will be the imposed straight line and the lower the calculated plasma volume. Values of 41 cc., 47 cc. and 61 cc. respectively per kilogram were calculated from curves IV, V and VI, all from a single experiment. Even when far smaller differences in the periods of observation are employed, plasma volumes calculated by the L.E.M. may vary significantly.

By what means, then, can a knowledge of the dye dilution in plasma be utilized for estimation of plasma volume? Since the relatively slowly-disappearing dye, T-1824, passes from the circulation in recognizable amounts during the mixing period, a method which accounts for such dye loss is desirable. It should be remembered, however, that even using an adequate expression for dye loss after uniform dye distribution, there is at present no proof of the assumption that dye loss will be accurately represented by this same expression during the mixing period. Nevertheless, since there is no direct experimental method available at present for testing the relation during this period, we are dependent upon extrapolation as the best present method of calculating the dye concentration at the moment of injection.

The solution, in the opinion of the authors, lies in determining a relationship between dye disappearance and time which will adequately express average dye loss over an extended period, so that a single straight line may be obtained which can be extrapolated to the time of injection from any period.

The clue to such a relationship was found in curves such as IV, V and VI of figure 3. When the data for various intervals after injection were plotted on appropriate scales it was seen that the curves were practically identical in shape. This observation, and the ratios between the co-ordinate scales of these graphs, indicated that the dye loss was proportional to the square root of the time after injection. The dye concentration c, at time t, is then given by

$$c = c_0 (1 - \sqrt{t/T}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where c_0 is the initial concentration and T is the time constant of the process. This equation was tested for its utility as a purely empirical device, and in the following discussion it will be called the "root extrapolation method," and designated by R.E.M.

The dye densities from 100 experiments[2] were accordingly replotted on the square root of time scale. The six experiments plotted linearly in figure 1 are shown in figure 2 plotted by the square root method. It may be seen that a single straight line suffices to represent average dye loss after an initial period of from three to ten minutes, regardless of whether the observation is continued for 100 minutes or for periods up to 24 hours. It is to be assumed that this single function will continue to describe dye disappearance for extended periods only where the physiological condition of the animal remains reasonably constant. It was found that during the longer experimental periods, however, certain procedures of convenience did not alter the straight line relationship. The dogs

[2] These include experiments performed by the authors, and others made available through the courtesy of Professor Gregersen.

were removed from the animal boards to their cages during prolonged intervals between sampling, they were permitted to move freely in their cages, to sleep, to drink water *ad libitum*, but undue muscular exercise and excitement were avoided.

Figure 4 shows experiment 107 plotted linearly and by the square root method. On the linear plot the three straight lines shown in curves IV, V and VI of figure 3 have been indicated. These serve to emphasize the increasing difficulty in the choice of a straight line of best fit when the period of observation is extended. The square root plot, on the other hand, establishes the choice of a line more conclusively with extension of the period of observation. In this experiment, where the distribution of points allows for some option in choice of a line, the extreme limits have been indicated in figure 4b. The plasma volumes calculated for these two extremes differ only by 2.13 per cent.

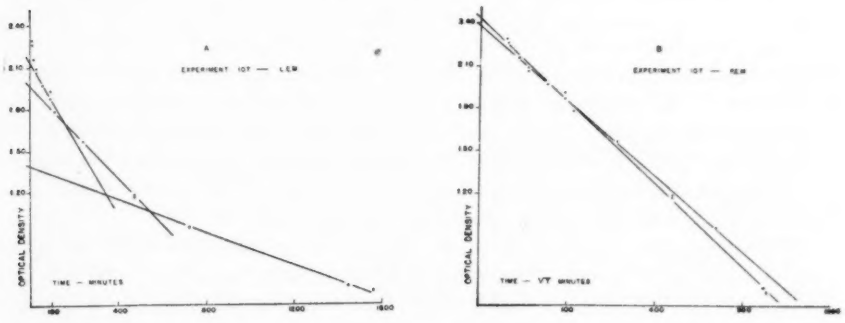

Fig. 4. A. Illustrates choice of line which may be drawn on a linear plot when the period of observation is extended.

B. The same data plotted as a function of the square root of time. Extremes of the choice of line are indicated.

Furthermore, when a square root curve, given by equation (3), is plotted on a linear time scale, the tangent is vertical at time $t = O$, so that, even without the complication of mixing, a linear plot of such a function is particularly difficult to extrapolate graphically to the initial concentration.

Plasma volume values for 100 experiments performed in these laboratories were calculated by both the L.E.M. and the R.E.M. The means and standard deviations for the two extrapolation methods were 52.6 $\pm$ 5.4 cc. per kilogram and 47.2 $\pm$ 5.3 cc. per kilogram respectively. There was great variability in the differences in individual values calculated by the two methods, hence values cannot be converted merely by application of a factor.

Standard deviations are approximately the same for the two series. Precision of this grade is attained with the L.E.M. only when the same or very similar time ranges are used for every experiment in the series. With the R.E.M. the precision will be the same whatever time interval within the first 24 hours is used for extrapolation.

The accuracy of the determination of the dye concentration at the moment of injection, and hence of the calculated plasma volume, necessarily depends upon the degree to which the average dye loss function employed represents dye loss during distribution.

If the square root function adequately describes average dye loss during the first three to ten minutes, then deviations of the points from the line within this period would indicate incomplete mixing. The reports of Graff, d'Esopo and Tillman (1931); Gibson, Keeley and Pijoan (1938) and Gilder, Muller and Phillips (1940) favor the view that a period of five to eight minutes is occupied by distribution of the dye. Observations by the authors confirm the view that mixing is complete within this period. If mixing is complete within two or three circulations, these deviations must depend on other factors (Robinow and Hamilton, 1940).

If the dye concentration were an exponential function of the time after injection as given by equation (1), we might have assumed that the dye loss was governed by a first order chemical reaction, or that the rate of dye loss from the blood stream was proportional to the concentration difference across a rather thin barrier. In the present case we turn to phenomena which involve the square root of the time, such as the "parabolic law" for the oxidation of metal surfaces, the "Schutz-Borissoff law" for enzyme kinetics, or the diffusion process described by the Fourier heat conduction equation. This latter process has interesting implications which cannot be discussed here.

Empirically, the equation (3) is an adequate representation of the data for periods from three to ten minutes up to 24 hours after injection. Within the range of the present data it may be seen that the disappearance curve is completely described by the constants c_0 and T.

It is obvious that equation (3) cannot be expected to describe the data for an indefinitely long time because c would become negative when $t = T$. Consequently the equation has additional terms which become important before $t = T$. The average value for T in the 100 experiments analyzed was found to be 36.4 hours (with the large standard deviation of ± 18.6 hours). These terms depend upon the mechanism and the geometry of the system and they have been calculated in several simple cases.

It is recognized that the concentration of dye in the blood is probably dependent upon several factors, such as: 1, the rate at which the dye, linked with albumin,[3] leaves the capillary bed and re-enters the circulation with the lymph; 2, the rate at which dye is excreted from the body, presumably through the activity of the liver; 3, the amount of dye held in readily staining tissues such as those of the lymph glands and kidneys, and whether such dye is available for later distribution. Mathematical functions used to describe the change in dye concentration over short or long periods may either represent the resultant of several processes or may express a limiting process.

We conclude that the time-concentration curve of the dye is best represented

[3] Rawson (in press) has shown that in presence of plasma proteins T-1824 is selectively linked with the serum albumin molecule.

as a function of the square root of time. Although this single function represents average dye disappearance during any period from ten minutes to twenty-four hours after injection, it cannot be stated dogmatically that this relation necessarily holds during the mixing period.

SUMMARY

1. The dye T-1824, when injected into the blood stream of the dog, disappears at a constantly diminishing rate.

2. Average dye loss cannot be adequately expressd by a single linear function for more than a very limited range of dye densities. As a consequence, deviation of the earlier points from such lines cannot be accepted as a criterion of incomplete distribution.

3. Values for plasma volume calculated from linear plots will vary with the period chosen for extrapolation, lower values characterizing determinations based upon periods shortly after dye injection.

4. Dye disappearance may be expressed adequately by a single straight line if dye densities are plotted as a function of the square root of time. This relationship maintains after the first five or ten minutes following injection and for any period up to twenty-four hours.

5. In contrast to the varying values obtained by extrapolation of different portions of the linear plot, the same value for plasma volume will be obtained during the first twenty-four hours whatever portion of the square root plot is used for extrapolation.

6. Plasma volumes calculated by the square root extrapolation method for any period between ten minutes and twenty-four hours average 10 per cent less than those calculated by the linear extrapolation method for periods between 30 and 120 minutes. The average percentage difference cannot be used as a factor for relating the two methods because the differences show considerable variability.

REFERENCES

ERLANGER, J. Physiol. Rev. **1:** 177, 1921.
GIBSON, J. G. AND W. A. EVANS. J. Clin. Investigation **16:** 301, 1937.
GIBSON, J. G., J. L. KEELEY AND M. PIJOAN. This Journal **121:** 800, 1938.
GILDER, H., O. H. MULLER AND R. A. PHILLIPS. This Journal **129:** 363, 1940.
GRAFF, S., D. A. D'ESOPO AND A. T. B. TILLMAN. Arch. Int. Med. **48:** 821, 1931.
GREGERSEN, M. I. AND J. D. STEWART. This Journal **125:** 142, 1939.
GREGERSEN, M. I. Macleod's Physiology in modern medicine. St. Louis, The C. V. Mosby Co., 1068, 1941.
KENNEDY, J. A. AND G. A. MILLIKAN. J. Physiol. **93:** 276, 1928.
KING, B. G., E. T. OPPENHEIMER AND K. S. COLE. Fed. Proc. **1** (II): 47, 1942.
RAWSON, R. A. In press.
ROBINOW, M. AND W. F. HAMILTON. This Journal **126:** P609, 1939.
ROBINOW, M. AND W. F. HAMILTON. Am. J. Dis. Child. **60:** 827, 1940.
SMITH, H. P. Johns Hopkins Hosp. Bull. **26:** 325, 1925.
SUNDERMAN, F. W. AND J. H. AUSTIN. This Journal **117:** 474, 1936.

DISTRIBUTION IN LEADS I, II AND III OF POTENTIALS APPLIED TO THE SURFACE OF THE HEART[1]

H. E. HOFF, L. H. NAHUM AND W. KAUFMAN[2]

From the Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Received for publication November 13, 1942

A variety of experimental evidence has permitted two conclusions concerning the nature of the electrocardiogram: 1. Excitation at the surface of the right ventricle is recorded in the three conventional leads of the electrocardiogram as a monophasic-like complex, directed upward, while activation of the surface of the left ventricle produces a similar complex of opposite direction. The electrocardiogram is produced by the summation of these two components. 2. Lead I records the summation of the anterior levocardiogram and the posterior dextrocardiogram, while lead III records the summation of the anterior dextrocardiogram and the posterior levocardiogram. Lead II is the summation of leads I and III (1–8).

As a further test of the validity of these conclusions a study was made of the distribution in leads I, II and III of direct potentials applied to the surface of the ventricles. By this method it was possible to determine *a*, the direction of electrocardiographic displacement due to negativity at the surface of the right or left ventricle, and *b*, the leads in which potential differences applied at the various surfaces of the ventricles are preponderantly recorded.

METHODS. Six dogs were employed, prepared as previously described (4). Pairs of circular tin electrodes 1 cm. in diameter were applied to the surface of the heart and were held in position by careful stitching through the epicardium or by the approximation of the pericardium. The electrodes were connected by fine insulated wires to a potentiometer across one or two dry cells. A commutator in the circuit made it possible to change the polarity at the electrodes. Potential differences with voltage low enough to avoid ventricular fibrillation were applied momentarily across these electrodes and the resultant beam deflections recorded in leads I, II and III of the electrocardiogram.

RESULTS. *Effect of negativity and positivity at right and left ventricular surfaces.* When one electrode was placed on the right ventricle, and the other on the left ventricle, negativity of the plate at the surface of the right ventricle and positivity of the plate at the surface of the left ventricle caused an upward deflection in all three standard leads. Reversal of polarity so that the plate at the surface of the left ventricle was negative resulted in a deflection of equal magnitude but downward in all three leads.

Distribution of potentials in the several leads. a. When the electrodes were placed at the centers of the anterior surface of the right ventricle and the pos-

[1] Supported by a grant from the Fluid Research Funds, Yale University School of Medicine.

[2] Fellow of the Dazian Foundation.

terior surface of the left ventricle, and potentials applied regardless of polarity, a minimum deflection was found in lead I, which was occasionally unmeasurable. Lead III, however, showed a large excursion and lead II showed the summation of leads I and III (fig. 1, A, B).

b. When potentials were applied across electrodes placed at the centers of the anterior surface of the left ventricle and the posterior surface of the right ven-

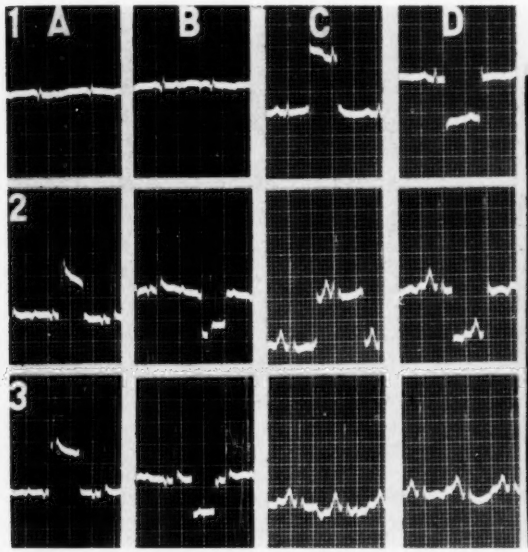

Fig. 1

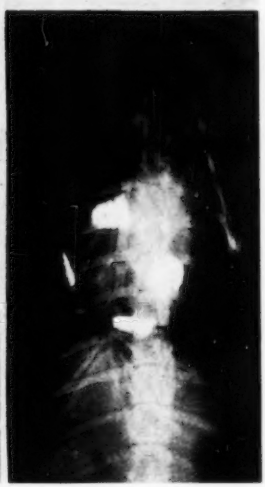

Fig. 2

Fig. 1. February 11, 1942. Dog. 10.0 kgm. Dial anesthesia. Electrocardiograms from leads I, II and III showing make and break of applied potential. A. Plates on anterior right and posterior left ventricles. Negative plate on right ventricle. B. Plates as in A, but polarity reversed. C. Plates on posterior right and anterior left ventricles. Negative plate on right ventricle. D. Same as C, but polarity reversed.

Fig. 2. March 4, 1942. Dog. 7.5 kgm. Dial anesthesia. Antero-posterior x-ray of dog with plates in position. Triangular plates were placed on the anterior surface of the right ventricle and on the posterior surface of the left ventricle. Electrocardiograms of applied potentials showed preponderant effects in lead III (see fig. 1, A and B). The circular plates were placed on the posterior surface of the right ventricle and the anterior surface of the left ventricle. Electrocardiograms showed preponderant effects in lead I when potentials were applied, as seen in fig. 1, C and D.

tricle, regardless of polarity lead III showed minimal or no deflections, while in lead I large ones were recorded. Lead II again recorded the summation of leads I and III (fig. 1, C, D).

c. When the paired electrodes were placed (*a*) at the centers of the anterior surfaces of the right and left ventricles, (*b*) at the lateral margins of the right and left ventricles, or (*c*) at the centers of the posterior surfaces of the right

and left ventricles and potentials applied, leads I and III showed approximately equal deflections, in the same direction.

d. When four electrodes were placed at the centers of the anterior and posterior surfaces of the right and left ventricles, and the two anterior plates made negative, lead I showed a downward and lead III an upward deflection. When the posterior electrodes were made negative, the deflection was upward in lead I and downward in lead III. In both instances, lead II recorded the summation of leads I and III.

Surface projection of the axes recorded in leads I and III. In the above experiments (*a*, *b*, *d*) in which one pair of plates was placed on the centers of the anterior right and posterior left ventricles and the other pair on the centers of the posterior right and anterior left ventricles and potential differences applied across them, it can be considered that two electrical axes were established, one passing through the centers of the anterior left and posterior right ventricles, and the other through the centers of the anterior right and posterior left ventricles.

To permit visualization of the anatomical orientation of these axes, thin steel rods were inserted through the heart joining the centers of the paired plates. The projection of the axis joining the anterior right and posterior left ventricles on the horizontal plane of the body was roughly parallel to the longitudinal axis of the body or to a line joining the base of the left leg and left arm. The projection in the horizontal plane of the axis joining the centers of the posterior right and anterior left ventricles was roughly parallel to the transverse axis of the body.

Another way of visualizing the anatomical orientation of these axes is seen in figure 2. This figure shows an x-ray film of the thorax with the plates in position at the approximate centers of the anterior and posterior surfaces of the two ventricles. The lines joining the paired plates closely parallel the longitudinal and transverse axes of the body.

Discussion. It is generally accepted that in irritable tissue an active region is electronegative to an inactive area. In the heart it has been proposed that electronegativity associated with excitation in the right ventricle produces a monophasic action potential recorded in the three conventional leads of the electrocardiogram as an upward deflection, and that electrical activity in the left ventricle produces a similar complex directed downward. The experiments recorded here have shown that negativity when physically applied at the surface of the right ventricle does in fact evoke an upward deflection, while negativity similarly applied at the surface of the left ventricle causes a downward deflection.

The distribution of potentials applied to the surface of the heart follows closely that of potentials generated by the heart itself, i.e., lead I records preponderantly the potential changes between the anterior left and posterior right ventricles, while lead III records the potential changes between the anterior right and posterior left ventricles. This similarity in distribution appears to be accounted for by the virtual identity of the electrical axes with the anatomical axes joining the centers of the respective surfaces which are recorded in leads I and III.

It was seen that the projection in the horizontal plane of the axis joining the centers of the anterior right and posterior left ventricles was roughly parallel to the longitudinal axis of the body. Potential changes created at points along this axis would be expected to influence preponderantly lead III, and to have a minimal effect in lead I. Similarly, the fact that the axis of the anterior left and posterior right ventricles has a projection on the frontal plane at or near a right angle to the longitudinal axis of the body, explains why changes along this axis should have a minimal effect in lead III and a maximal influence in lead I.

It should be pointed out that such anatomical relationships vary in different specimens of the same species, in different species, and in each individual because of change in the position of the heart, etc. It should be expected, therefore, that the electrical representation of cardiac surfaces in leads I and III may be preponderant and not exclusive. This has been the case in these experiments as well as in those of different types which preceded them.

The observation that, when the anterior surfaces of both right and left ventricles are made electronegative simultaneously, an upward deflection is registered in lead I and a downward deflection in lead III, while opposite changes occur when the posterior surfaces are made negative, is a counterpart of earlier observations on the nature of leads I and III (5). It confirms conclusions drawn from them, namely, that the anterior surface of the right ventricle and the posterior surface of the left ventricle are represented preponderantly in lead III, while in lead I are recorded the electrical events at the surfaces of the anterior left ventricle and the posterior right ventricle.

SUMMARY

1. Potential differences applied across the surfaces of the right and left ventricles cause an upward deflection in the standard leads of the electrocardiogram when the negative electrode is on the right ventricle, and a downward deflection when the negative plate is on the left ventricle.

2. Potential differences applied across the centers of the anterior surface of the right ventricle and the posterior surface of the left ventricle affect preponderantly lead III of the electrocardiogram. The projection in the horizontal plane of the line joining these centers is roughly parallel to the longitudinal axis of the body.

3. Potential differences applied across the centers of the anterior surface of the left ventricle and the posterior surface of the right ventricle are recorded preponderantly in lead I. The projection in the horizontal plane of the line joining these centers is roughly parallel to the transverse axis of the body.

REFERENCES

(1) Hoff, H. E., L. H. Nahum and B. Kisch. This Journal **131:** 687, 1941.
(2) Nahum, L. H., H. E. Hoff and B. Kisch. This Journal **131:** 693, 1941.
(3) Hoff, H. E., and L. H. Nahum. This Journal **131:** 700, 1941.
(4) Nahum, L. H., H. E. Hoff and W. Kaufman. This Journal **134:** 384, 1941.
(5) Hoff, H. E., L. H. Nahum and W. Kaufman. This Journal **134:** 390, 1941.
(6) Nahum, L. H., H. E. Hoff and W. Kaufman. This Journal **134:** 398, 1941.
(7) Hoff, H. E., L. H. Nahum and W. Kaufman. This Journal **135:** 752, 1941–42.
(8) Nahum, L. H., H. E. Hoff and W. Kaufman. This Journal **136:** 726, 1942.

NINHYDRIN, CRYSTALLINE PAPAIN AND FIBRIN CLOT

JOHN H. FERGUSON AND PAUL H. RALPH[1]

From the Departments of Pharmacology and Zoology, University of Michigan, Ann Arbor

Received for publication November 6, 1942

Eagle and Harris (1) discovered that *papain* "acts directly on fibrinogen to form a fibrillar gel resembling fibrin." This has been confirmed (2) with a cyanide-activated *crystalline* papain enzyme, kindly supplied by Dr. A. K. Balls, U. S. Department of Agriculture. The same preparation was used in the present observations. A recent brief report (3) claims that fibrinogen can be clotted by the simple chemical agent *ninhydrin* (1,2,3-indantrione hydrate). In view of the importance of these data in the interpretation of the fundamental mechanisms of fibrin formation, they have been re-investigated with the aid of dark-field microscopy (cf. Stübel, 1914; Howell, 1914), in comparison with ordinary thrombic clots. Additional experiments by established methods (4) evaluate the possible rôle of ninhydrin in relation to blood-clotting mechanisms.

REAGENTS AND METHODS. Prothrombin and fibrinogen were prepared from citrated dog plasma and used according to the routine methods for experimental analysis of coagulation mechanisms previously described (4). The 0.9 per cent NaCl, used throughout as vehicle and diluent, was saturated with thymol. A 1:100 dilution of Parfentjev's (5) "rabbit clotting globulin" (Lederle Lab.) provided an excellent tryptase-free thrombin (T_G).

A lyophilized human fibrinogen (cf. 6) of high purity was supplied through the courtesy of Dr. E. J. Cohn (Harvard). It yielded clear, stable solutions completely free from thrombin and prothrombin and containing only a mere trace of serum-tryptase which caused "spontaneous" fibrinolysis of thrombic (T_G) clots in several days at room temperature. The fibrinogen was made up in phosphate buffer solution at pH = 7.7, somewhat diluted. Final fibrinogen concentration = 0.8 mgm. per cc. Crystalline papain: v. supra. Ninhydrin: a reliable German crystalline preparation was dissolved in distilled water to form a "stock" solution containing 20 mgm. per cc. With the larger quantities of ninhydrin the pH of the somewhat inadequately buffered fibrinogen was shifted almost to neutrality (phenol red indicator).

Data. Thrombin (T_G) and crystalline papain gave solid gels within a few seconds after adding to the fibrinogen solutions. The respective dark-field appearances are seen in figure 1, A and B. The typical fibrin needle-like meshwork appears to be identical in the two cases. The photomicrographs were made from a preparation of dog fibrinogen, nearly, but not quite, free from prothrombin. Exactly similar appearances were also obtained with the completely prothrombin-free human fibrinogen. Ninhydrin was likewise tested on both dog and human fibrinogen, the photomicrographs (fig. 1, C and D) being obtained with the cited dog material. Numerous strengths of ninhydrin were used, from contact of the fibrinogen (1 cc.) with undissolved crystals down to a (final) ninhydrin concentration of $\frac{1}{6}$ mgm. per cc. The mixtures invariably be-

[1] Dr. P. H. Ralph now holds a Horace H. Rackham Postgraduate Fellowship in the Department of Medicine, Ohio State University.

came turbid, in 1 to 2 minutes with the strongest ninhydrin and only after 1½ hours with the weakest strength employed. In all tubes a flocculent granular deposit formed later but at no time did any of the tests yield a gel sufficient to permit of inversion of the (11 mm.) tubes. However, the stronger concentrations developed a slimy supernatant that bore a crude resemblance to a "clot." Under the dark-field, two appearances were noted and are well illustrated in the photomicrographs.

The first (C) was a refractile granular deposit resembling that seen when fibrinogen or other proteins are "denatured" by heating or various flocculating agents. Sometimes the granules were larger, ovoid and budding (very like yeasts) or rounded and wrinkled, of size and appearance suggestive of crenated red blood-cells.

The second appearance (D) was an interesting thread-like formation (like mucus), the units of which were typically very long, straight, and arranged in parallel bundles. Sometimes they showed spiculated branching, and not in-

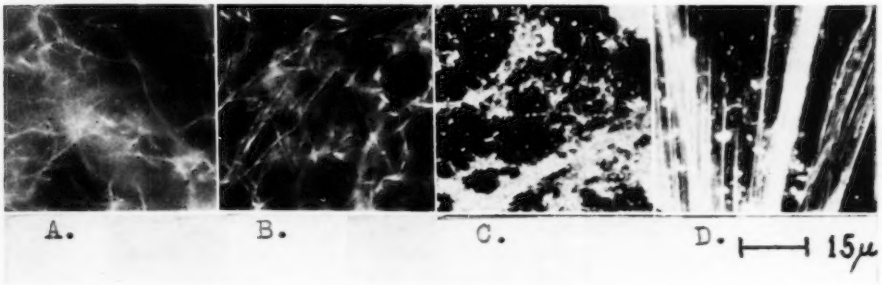

Fig. 1. Dark-field microscopy (oil-immersion lens) of fibrinogen (dog) mixed with: A, tryptase-free thrombin; B, crystalline papain; C; D, ninhydrin.

frequently there was evident a double outline and sometimes a series of varicose swellings.

It is obvious that these appearances differ widely from the typical fibrin meshwork and it is, therefore, concluded that ninhydrin is not in any real sense a thrombin-like substance capable of converting fibrinogen into true fibrin. The possibility remains that it might assist in thrombin formation in cruder systems containing thrombin precursors, in amounts greater than the traces identified in the cited dog fibrinogen. The following studies were made of the effects of ninhydrin on the isolated clotting mechanisms.

Ninhydrin and the thrombin-fibrinogen interaction (21.5°C.): Control: 1.0 cc. human fibrinogen + 0.25 cc. dist. water + 0.5 cc. thrombin (T_G) = clot in 28 sec. *Test:* 1.0 cc. human fibrinogen + 0.25 cc. ninhydrin + 0.5 cc. thrombin (T_G) = clot in 37 sec. The "stock" ninhydrin solution merely caused an unimportant delay in clotting which is attributable to a slight increase in acidity. A similar minor "second phase" effect can also account for the small differences in the earlier tests of A and B in table 1 (v. infra). Ninhydrin, therefore, is without significant effect on the thrombin-fibrinogen interaction.

Ninhydrin and the conversion of prothrombin to thrombin. Thrombic mixtures, each containing 4 cc. prothrombin and 0.9 per cent saline (as necessary) to make 5 cc., included the following, respective, *activators*: A. tissue thromboplastin (0.25 cc. dil. saline extract of frozen dog-brain) + 0.25 cc. N/10 $CaCl_2$; B. 0.25 cc. br. extr. + 0.25 cc. $CaCl_2$ + 0.25 cc. "stock" ninhydrin; C. 0.25 cc. $CaCl_2$ (alone); D. 0.25 cc. $CaCl_2$ + 0.25 cc. ninhydrin; E. 0.25 cc. ninhydrin (alone). Table 1 summarizes the activation tests, conducted at 21.5°C., pH = 7.7. Clotting-times are for 0.5 cc. samples of thrombic mixture, removed after the (incubation) periods cited and mixed with 1.0 cc. of a human fibrinogen solution, which control tests (*sans* prothrombin) proved to be completely free from prothrombin.

The ninhydrin-containing mixtures D and E developed a turbidity after 1½ hours and a subsequent fine granular deposit, but at no time was there any true clot formation. The extremely poor activation by calcium alone (C) shows that the prothrombin solution was all but free from thromboplastic factors. There

TABLE 1

Effect of ninhydrin on conversion of prothrombin to thrombin

Temp. = 21.5°C., pH = 7.7. Clotting-times (sec.) for 0.5 cc. thrombic mixture + 1.0 cc. prothrombin-free fibrinogen.

	INCUBATION PERIOD							
	5 min.	10 min.	20 min.	30 min	1 hr.	1½ hr.	2 hr.	13 hr.
1. Pro. + brain extr. + Ca.	23″	18″	15″	15″	18″	21″	22″	25″
2. Pro. + br. ext. + Ca + Nin.	34″	20″	16½″	16½″	26″	38″	56″	320″
3. Pro. + Ca.	trace	trace	trace	trace	trace	trace	±	+ 35
	24 hr.	24 hr.	24 hr.	24 hr.	12 hr.	12 hr.	12 hr.	min.
4. Pro. + Ca + Nin.*	∞	∞	∞	∞	∞	∞	∞	∞
5. Pro. + Nin.*	∞	∞	∞	∞	∞	∞	∞	∞

* Flocculent turbidity in 1½ hr.

is not the slightest evidence that ninhydrin can serve as a thromboplastic factor, alone or with calcium. The action of ninhydrin (B) during the first 20–30 min. activation phase in a complete thrombic system (prothrombin + Ca + added thromboplastin) consists of a negligible lessening of the apparent thrombic potency, especially in the early stages. This amounts only to a 1½ sec. difference at the "optimum" and in view of the above-noted minor second-phase effects, due to difficulty in controlling the acidifying tendency of the ninhydrin, may be dismissed as an inconsequential delay in the thrombin-fibrinogen interaction. It may be concluded that ninhydrin is without significant effect on the first phase of clotting also.

Ninhydrin and thrombinolysis; fibrinolysis. A comparison of A and B in the later stages (up to 13 hrs.) is interesting. Thrombin A was only very slightly unstable, due probably to a very small trace of thrombinolytic factor (? tryptase) in the crude brain thromboplastin. Thrombin B is definitely less stable. The clots of series A underwent fibrinolysis in 3 to 4 days, while those of series B

resisted lysis for 2 to 3 days longer. They were also more turbid. It may be concluded that ninhydrin, in common with innumerable other agents, alters the colloidal architecture of proteins and thus modifies the lytic phenomena, which are only incidental to clotting.

SUMMARY

Dark-field microscopy reveals the similar appearance of fibrin clots formed in prothrombin-free fibrinogen by 1, tryptase-free thrombin; 2, crystalline papain. The latter clots, unlike the former, undergo subsequent fibrinolysis. The dark-field appearances of the "pseudo-clots" formed by the action of ninhydrin on fibrinogen solutions, are quite different. An analysis of the behavior of ninhydrin in experimentally isolated clotting systems shows it to be without significant effect upon the fundamental coagulation mechanisms.

REFERENCES

(1) Eagle, H. and T. N. Harris. J. Gen. Physiol. **20:** 543, 1937.
(2) Ferguson, J. H. and A. J. Glazko. J. Lab. and Clin. Med. **26:** 1559, 1941.
(3) Chargaff, E. and M. Ziff. J. Biol. Chem. **138:** 787, 1941.
(4) Ferguson, J. H. J. Lab. and Clin. Med. **24:** 273, 1938.
(5) Parfentjev, I. A. Am. J. Med. Sci. **202:** 578, 1941.
(6) Ferguson, J. H. and B. N. Erickson. Proc. Soc. Exper. Biol. and Med. **40:** 425, 1939.

THE INFLUENCE OF THYROID, DINITROPHENOL AND SWIMMING ON THE GLYCOGEN AND PHOSPHOCREATINE LEVEL OF THE RAT HEART IN RELATION TO CARDIAC HYPERTROPHY

WALTER B. SHELLEY, CHARLES F. CODE[1] AND MAURICE B. VISSCHER

From The Department of Physiology, University of Minnesota, Minneapolis

Received for publication September 8, 1942

This investigation was undertaken in an attempt to gain insight into the chemical processes concerned in the development of cardiac hypertrophy. All observations were made on albino rats. It has been shown that this species develops an increased heart weight promptly upon feeding desiccated thyroid gland or thyroxin (1). This convenient method of altering cardiac size was used in the first series of experiments. In the second series of observations, attempts were made to influence the weight of the heart by the administration of dinitrophenol, and in the final series of experiments increased cardiac weight was induced by exercise.

Throughout the investigation all animals received a diet of Purina fox chow. At the termination of each experiment the rats were anesthetized by the intraperitoneal injection of pentobarbital sodium (50 mgm. per kgm. body weight).

I. *Hyperthyroid Cardiac Hypertrophy.* Previous investigators in the field of cardiac hypertrophy have not concerned themselves with the chemical changes in the *hyperthyroid* hypertrophied heart. The present experiments were designed to study the cardiac glycogen and phosphocreatine levels during the process of hyperthyroid hypertrophy.

1. *Glycogen concentration.* The glycogen concentration in the hypertrophied hyperthyroid heart has not been investigated, although Lawrence (2) and at least ten other workers have shown that in the acute thyrotoxic state, the cardiac glycogen levels are uniformly low.

In the present study, hyperthyroidism was induced by feeding dried thyroid glands (0.3 per cent organic I_2). The hearts, removed essentially according to the method of Evans (3), were analyzed for glycogen using a slight modification of Good's (4) procedure. The extent of cardiac hypertrophy was measured by comparing the heart weight/final body weight ratios (H.W./F.B.W.) of experimental and control animals. All analyses were made on 24-hour fasted animals.

The heart weight/body weight ratios and cardiac glycogen concentrations in all of the normal control rats studied were averaged. Seventy-four normal rats had a H.W./F.B.W. ranging from 2.6 to 4.5 $\times 10^{-3}$, averaging 3.2 $\times 10^{-3}$. In 36 normal rats the cardiac glycogen varied from 380 to 660 mgm. per cent, with an average value of 494 mgm. per cent. The standard deviation was found to be ± 68 mgm. per cent.

Feeding dried thyroid gland in daily doses of 1.0, 0.5 and 0.25 mgm. per gram of body weight, or feeding a diet containing 0.3 or 0.7 per cent thyroid to 102

[1] Present address—Mayo Foundation, Rochester, Minnesota.

rats for periods varying from one week to six months resulted in progressive cardiac hypertrophy and progressive glycogen loss. The maximal H.W./F.B.W. was 8.1 $\times$ 10^{-3}, and the minimal glycogen value was 41 mgm. per cent. The degree of hypertrophy and glycogen loss was directly proportional to the size of the daily dose and the length of period during which it was administered. Apparently in the progressive development of hyperthyroid cardiac hypertrophy, no compensatory mechanism restored the glycogen in the heart to normal levels.

a. *Effect of age* (fig. 1): A series of observations was made to determine the effect of age on the response of the heart to thyroid feeding. Young (19 rats, 7 weeks of age at the start), adult (11 rats, 6 months of age), and senile (10 rats over 2 years of age) groups have been simultaneously studied. Each group received the same daily dose of thyroid, viz., 0.25

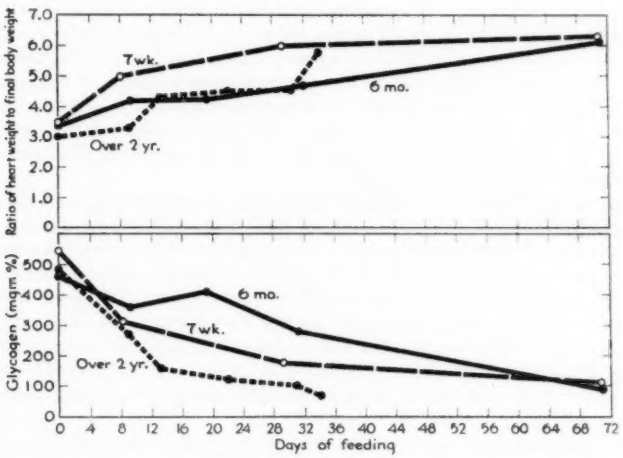

Fig. 1. Cardiac glycogen levels and H.W./F.B.W. $\times$ 10^3 in thyroid-fed rats of varying ages.

Daily dose of thyroid: 0.25 mgm./gram of body weight.

mgm. per gram of body weight. Animals were killed at about one week, one month, and two months after beginning thyroid feedings. In the senile group none of the animals lived for two months, indicating some decrease in resistance to thyroid feeding in old rats. The three groups showed a comparable degree of cardiac hypertrophy. The results indicate that the development of the low cardiac glycogen concentration in the hyperthyroid hypertrophied rat heart is independent of the age of the animal. In all cases the cardiac glycogen was low in the presence of hyperthyroid hypertrophy.

b. *Effect of the technique used in removing the heart.* Electrocardiographic measurement of the heart rate in six hyperthyroid rats revealed a heart rate varying from 550 to 650 beats per minute, which is about twice the value found in normal rats of the same age. This acceleration of the energetic processes should cause an increased oxygen requirement of the heart. Chang (5) has shown that the cardiac glycogen in the normal rat is rapidly depleted by anoxia and adrenalin. Because of these factors it appeared possible that the low glycogen concentration observed in the hyperthyroid heart resulted from hypersensitivity to the anoxia and adrenalin incident to the removal of the heart which required three to five seconds. In an attempt to eliminate the effects of anesthesia and surgery, and there-

by anoxia and adrenalin liberation, the following experiments were performed: A device was made in which it was possible to instantaneously sacrifice and transect the untreated animal with a single stroke through the chest. The exposed heart was immediately transferred to hot potassium hydroxide solution. In three normal rats so treated the cardiac glycogen level averaged 465 mgm. per cent, which agrees satisfactorily with the value obtained above. The hearts of three hyperthyroid rats (fed 0.5 mgm. thyroid per gram of body weight daily for 17 days) when removed by this procedure, showed an average level of 110 mgm. per cent, which again is in the same range as the values obtained using the standard method.

In order to eliminate possible effects of adrenalin and to shorten the anoxial period during removal the following method of removing the heart was designed. Under ether anesthesia the rats were bilaterally adrenalectomized. Seven to ten hours later the rat was anesthetized with pentobarbital sodium, artificial respiration established, and a single midsternal incision made. After 15 to 20 minutes a mixture of ether and solid carbon dioxide was poured on the heart. Nine normal rat hearts removed in this way showed an average glycogen concentration of 497 mgm. per cent. Using this technique the cardiac glycogen values in the hyperthyroid animals were again low. Five rats, fed a 2 per cent thyroid diet for six days, showed an average glycogen concentration of 178 mgm. per cent. The mean H.W./F.B.W. was 4.5×10^{-3}. These two experiments support the view that thyroxin has a specific glycogenolytic action, and that throughout this series of experiments neither the anesthesia nor the temporary anoxia associated with removal of the hearts appreciably influenced the results.

2. *Phosphocreatine* (table 1). No studies were found on the phosphocreatine level in the hypertrophied hyperthyroid heart. As in the case of glycogen, investigators have been interested in the acute hyperthyroid state in which cardiac hypertrophy is absent. Schumann (6) studying rats found variations in normal levels of cardiac phosphocreatine of from 9.9 to 13.9 averaging 11.0 mgm. per cent P. When 4 mgm. of thyroxin were given for a period of four days he found the level dropped to 4.8 mgm. per cent. Chanutin (7) showed that a 10 per cent creatine diet fed for two days elevated the normal rat heart creatine concentration from the control value of 217 mgm. per cent to 282 mgm. per cent. The effect of creatine feeding on the level of phosphocreatine in hyperthyroid hearts has not been reported.

The following experiments were undertaken to determine, first, the phosphocreatine concentration in *hypertrophied* hyperthyroid rat hearts, and second, the effect of feeding creatine upon the level of phosphocreatine in such hearts. The analytical procedure followed was that of Eggletons (8), using an Evelyn photo-electric colorimeter. It was found necessary both to freeze the hearts immediately in an ether-solid CO_2 mixture and to carry out the extraction at very low temperatures. The results in ten normal rat hearts ranged from 10.0 mgm. per cent to 14.5 mgm. per cent of phosphocreatine phosphorus with a mean of 12.0 mgm. per cent (table 1).

The rats, individually caged, received identical amounts of food. Two groups of rats fed a diet containing 0.3 per cent thyroid for 18 and 20 days showed cardiac hypertrophy. In the first group, with a H.W./F.B.W. of 4.3×10^{-3}, the phosphocreatine concentration was 4.9 mgm. per cent (table 1). In the second group with a marked degree of hypertrophy, H.W./F.B.W., 6.2×10^{-3}, the phosphocreatine level was 5.9 mgm. per cent. It is clear that the low levels of cardiac phosphocreatine, as well as glycogen, found in acute hyperthyroidism persisted during and after the development of cardiac hypertrophy.

A third group of five rats were maintained on a diet containing 0.3 per cent thyroid and 6.0 per cent creatine for 18 days. The average phosphocreatine concentration was 3.9 mgm. per cent as compared to a control group, receiving thyroid but no creatine, with an average of 4.9 mgm. per cent. A fourth group received a 0.3 per cent thyroid diet for 20 days, a 10 per cent creatine diet being substituted on the last day. The phosphocreatine level was on the average 1 mgm. per cent above the average control value of 5.9 mgm. per cent. It was concluded that creatine feeding did not significantly alter the cardiac phosphocreatine level of the hyperthyroid hypertrophied rat heart.

II. *Attempts to Produce Cardiac Hypertrophy by Slowly Absorbed Dinitrophenol.* Taussig (9), in experiments on rats, administered 10 to 15 mgm. of dinitrophenol per kilogram body weight twice a day for periods varying from 2 to 17 days. He found the cardiac glycogen concentration to be normal. Fatal doses produced a reduction in cardiac glycogen which he suggested was probably due to anoxemia. Wesselow (11) administered 50 mgm. of dinitrophenol daily in the food for one month to 200 gram rats in an unsuccessful attempt to produce cardiac hypertrophy. To determine if parenterally administered dinitrophenol is also ineffective in producing hypertrophy, the following experiments were conducted.

Dinitrophenol was incorporated into the beeswax-mineral oil mixture which

TABLE 1

Phosphocreatine concentration in the normal and hyperthyroid hypertrophied rat heart

DIET	F.B.W.	$\frac{\text{H.W.}}{\text{F.B.W.}} \times 10^3$	PHOSPHOCREATINE (MGM. PER CENT P)	DIET	F.B.W.	$\frac{\text{H.W.}}{\text{F.B.W.}} \times 10^3$	PHOSPHOCREATINE (MGM. PER CENT P)
Control	260	3.8	12.0	0.3% thyroid, 20 days	260	5.5	6.2
0.3 per cent thyroid, 18 days	310	3.7	4.3		315	4.9	5.9
	290	4.8	5.2		220	6.6	6.4
	310	4.5	4.5		170	8.0	5.1
	310	4.0	5.1				
	310	4.3	5.2				
Average		4.3	4.9	Average		6.2	5.9
0.3 per cent thyroid, 6.0 per cent creatine, 18 days	320	3.7	2.6	0.3% thyroid 20 days + 10% creatine last day	250	5.1	6.2
	270	5.8	5.1		250	5.3	7.5
	285	4.8	4.7		230	5.4	6.9
	285	4.2	2.5				
	285	5.0	4.5				
Average		4.7	3.9	Average		5.3	6.9

Code and Varco (11) found to be satisfactory for delaying histamine absorption. Six rats were each given 275 mgm. of 2-4 dinitrophenol subcutaneously in divided doses over a period of 16 days. No significant cardiac hypertrophy resulted, the average H.W./F.B.W. being 3.7×10^{-3}. The animals showed a 15 per cent loss in weight. The cardiac glycogen levels were essentially normal, the mean being 425 mgm. per cent, with a range from 275 to 531 mgm. per cent.

III. *Glycogen Concentration in Hypertrophied Hearts of Swimming Rats (table 2).* Kirch (12) has demonstrated in the rat that swimming can result in cardiac hypertrophy but no chemical analyses of such heart muscle have been reported. Accordingly the following studies were made to determine if the concentration of glycogen was altered by the development of this type of cardiac hypertrophy.

Young male rats (60–80 grams) were selected for this experiment. These animals were divided into six groups, all receiving the same diet. The first two groups were unexercised and served as controls. The remaining groups (groups

3, 4, 5, and 6) were put in a water bath at 25°C. for an average of 3.3 hours per day. After periods of 50 and 60 days the animals were sacrificed under pentobarbital with or without a previous 24-hour fast.

The hearts of the exercised animals were consistently heavier than those of the control animals. The heart weight: body weight ratio was significantly increased in the swimmers and this was not due to a loss of body weight. In

TABLE 2

Glycogen concentrations in hypertrophied hearts of swimming rats

GROUP	F.B.W.	H.W./F.B.W. × 10³	GLYCOGEN (mgm. per cent)	GROUP	F.B.W.	H.W./F.B.W. × 10³	GLYCOGEN (mgm. per cent)
1. Controls (fasted 24 hrs.)	160	4.0	444	4. Swimmers (not fasted) 60 days. 3.3 hrs./day. Killed 1–2 hrs. after swim	190	4.7	434
	210	3.4	531		184	4.4	556
	160	3.7	470		170	5.1	652
	220	3.5	565		150	5.7	648
					160	5.5	690
Average		3.6	502	Average		5.3	596
2. Controls (not fasted)	160	3.8	413	5. Swimmers (not fasted) 60 days. 3.3 hrs./day. Killed 24 hrs. after swim	180	4.6	510
	175	3.7	391		180	4.4	432
	190	4.3	550		180	4.5	388
	165	3.6	635		150	5.5	467
	150	4.5	485		120	4.6	482
	135	4.2	605		190	4.2	513
	180	3.7	412				
Average		3.9	498	Average		4.6	465
3. Swimmers (fasted 24 hrs.) 50 days. 3.3 hours/day. Killed 24 hrs. after swim	150	5.5	1120	6. Swimmers (not fasted) 50 days. 3.2 hrs./day. Killed 24 hrs. after swim	180	4.3	859
	180	5.1	1300		180	5.0	695
	140	5.2	1345		200	4.7	662
	165	5.3	790		200	4.4	671
	160	5.4	819		175	4.8	731
	145	5.5	1305				
Average		5.3	1013	Average		4.6	723

contrast to the results obtained with hyperthyroid hypertrophy, the glycogen concentration in the hearts of the exercised animals was normal or increased. In the swimmers (group 3) which had been fasted and rested for 24 hours prior to killing, the cardiac glycogen concentration averaged twice as high as normal. In the rats (group 4) killed 1 to 2 hours after swimming without fasting, the cardiac glycogen was normal. Conflicting results were obtained in groups 5 and 6. In both of these groups the rats were not fasted and were killed 24 hours after swimming. Group 5 showed essentially normal glycogen values while group 6 showed elevated levels. No explanation can be offered at the present

time for this variance. It has been concluded that in young rats swimming can produce hypertrophied hearts which show normal or increased glycogen concentrations 1 to 24 hours after swimming.

COMMENT. The experiments in which cardiac hypertrophy occurred in response to thyroid feeding make an interesting contrast to those in which hypertrophy occurred in response to exercise. When thyroid was fed over a prolonged period, the rat developed marked cardiac hypertrophy. When the rat was exercised each day for about three hours a moderate degree of hypertrophy occurred. In the thyroid-fed rat the cardiac glycogen fell to a low level and remained low throughout the entire experiment. The exercised animals were killed after a 1 to 24-hour rest and the glycogen level was then equal to or above the control levels. In the thyroid-fed rats the stimulating effects of the ingested thyroid acted continuously throughout the entire period of observation while in the exercised rats the metabolic stimulant was applied for only about three hours of each day. The glycogen level in the hearts of the exercised animals may have been low at some time during the three-hour period of exercise, but it was not tested. Actually, the exercised animals which had been fasted and rested for 24 hours showed the highest glycogen levels which we have encountered in any rat heart. It is important to note that no exercise studies were made in the adult rat which may show a different response.

In the experiments in which thyroid was fed it seemed there might be a relation between low cardiac glycogen levels and the development of cardiac hypertrophy. When the thyroid was fed, low glycogen concentrations in the heart preceded and accompanied the development of hypertrophy. During prolonged thyroid feeding the cardiac glycogen concentration remained low. Thus, in these experiments the progressive development and maintenance of hyperthyroid cardiac hypertrophy was not accompanied by a compensatory restoration of the glycogen in the heart.

SUMMARY

1. Both glycogen and phosphocreatine were found to be present in low concentrations in the *hypertrophied* hyperthyroid rat heart.

2. Dinitrophenol administered subcutaneously for a period of two weeks failed to produce an increase in the heart weight of rats.

3. Young rats which had been swimming 3.3 hours daily for about 2 months showed moderate cardiac hypertrophy. When determined 1 to 24 hours after swimming the cardiac glycogen was normal or elevated. When the animals had been fasted and rested 24 hours after swimming the cardiac glycogen concentration averaged twice as high as normal.

REFERENCES

(1) CAMERON, A. T. AND J. CARMICHAEL. J. Biol. Chem. **45:** 69, 1920.
(2) LAWRENCE, R. D. AND R. A. MCCANCE. Biochem. J. **25:** 570, 1931.
(3) EVANS, G. J. Physiol. **82:** 468, 1934.
(4) GOOD, C. A., H. KRAMER AND M. SOMOGYI, J. Biol. Chem. **100:** 485, 1933.

(5) CHANG, I. Quart. J. Exper. Physiol. **26:** 286, 1937.
(6) SCHUMANN, H. Ztschr. f. d. gesammt. Exper. Med. **105:** 577, 1939.
(7) CHANUTIN, A. AND N. SILVETTE. J. Biol. Chem. **80:** 589, 1928.
(8) EGGLETON, G. P. AND P. EGGLETON. J. Physiol. **68:** 183, 1929.
(9) TAUSSIG, B. J. Pharmacol. and Exper. Therap. **56:** 228, 1936.
(10) WESSELOW, O. L. AND W. J. GRIFFITHS. Brith. J. Exper. Path. **19:** 347, 1938.
(11) CODE, C. F. AND R. L. VARCO. Proc. Soc. Exper. Biol. and Med. **44:** 475, 1940.
(12) KIRCH, E. AND W. GRUNBAUER. Beitrag. Path. Anat. u. Allg. Path. **100:** 354, 1938.

EFFECTS ON MAN OF SEVERE OXYGEN LACK

S. M. HORVATH,[1] D. B. DILL[2] AND W. CORWIN[3]

From The Fatigue Laboratory, Harvard University, Boston, Massachusetts; The Aero Medical Research Laboratory, Wright Field, Dayton, Ohio; The Metropolitan State Hospital, Waltham, Massachusetts

Received for publication November 2, 1942

Interest in the response of schizophrenic patients to oxygen lack has existed for several years. Experience with various shock therapies has brought forth the view that their effects may be related to the induction of cerebral anoxia. This appears to be the case in the insulin shock treatment of Sakel (1) and in the nitrogen breathing treatment introduced by Himwich and associates (2). The rationale of the anoxia therapy used in our investigation is being presented by Corwin and Horvath elsewhere (3) and requires only brief mention here.

Since the results in any shock treatment depend on the duration as well as the intensity of the stimulus, we have employed mixtures of nitrogen and oxygen low enough in oxygen to produce unconsciousness and yet capable of sustaining life for many minutes. The intensity and duration of the stimulus was varied to suit the individual case. The principal gas mixtures used contained 4.2, 5.2 or 6.0 per cent oxygen. The duration of exposure to these mixtures varied from 3 to 16 minutes, 6 to 21 minutes and 4 to 15 minutes, respectively. Unconsciousness frequently terminated those treatments that involved either 4.2 or 5.2 per cent oxygen. It was rarely observed during the time intervals indicated with 6.0 per cent oxygen. In some instances there was an induction period during which the patient breathed 14 per cent oxygen. Recovery was effected usually by abruptly shifting from the gas mixture to air while on occasions 14 per cent oxygen was supplied during the first stage of recovery. Male patients were used as described in table 1.

The observations made include: *a*. Measurement of respiratory volume and rate before, throughout and after the anoxic period. *b*. Sampling and analysis of expired air before, during and after the anoxic period. *c*. Sampling and analysis of arterial blood before, during and after the anoxic period. *d*. Hematological responses. *e*. Pulse and blood pressure. *f*. Electrocardiograph. *g*. Mental state of the patient. Observations *a* to *c* provide the principal factual data on which this report is based.

RESULTS. The measurements of respiratory volume in those treatments involving 6.0, 5.2 and 4.2 per cent oxygen are shown in figures 1 to 3, respectively. In the preliminary period, hyperventilation was frequently observed. An unexcited person at rest will breathe from 5 to 8 liters per minute: the fact that some of these patients reached values as high as 15 liters per minute, the

[1] 1st Lt., Sn. C.
[2] Lt. Colonel, A. C.
[3] Captain, M. C.

average being about 10, is evidence of excitement and/or physical activity. Some remained quiet and unexcited, others were quiet but tense while a few were disturbed and resistant. Every possible precaution was taken to keep the face mask in place throughout but some tests failed because this was impossible.

There are several notable features of the results. There was invariably an

TABLE 1

Description of patients

The diagnosis was dementia praecox. The first seven were catatonic, the others hebephrenic.

SUBJECT	AGE	HEIGHT	WEIGHT	LENGTH OF HOSPITALIZATION
	years	*inches*	*pounds*	*years*
B. H. E.	26	66	122	8.0
C. J. G.	25	66	122	3.5
S. J. A.	27	66	176	8.0
C. W. A.	34	70	156	8.4
D. C.	32	71	185	6.0
D. F. W.	24	70	129	6.7
H. C. N.	23	71	128	8.5
M. D. R.	28	65	131	4.5
P. F.	34	67	140	16.7
Q. B. J.	33	66	120	5.6
C. J. H.	31	72	160	10.8

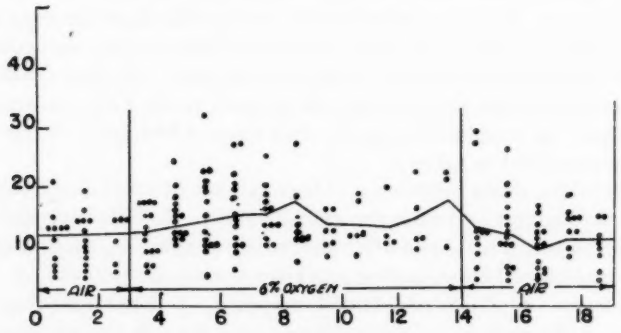

Fig. 1. Pulmonary ventilation in liters per minute before, during, and after breathing 6.0 per cent oxygen. The curve corresponds to the average response. Time is in minutes.

increase in pulmonary ventilation within two minutes after starting to breathe 4.2 per cent oxygen. The general trend throughout the first five minutes of anoxia was upwards, reaching, on the average, 30 liters per minute and in one case, during the sixth minute of anoxia, 65 liters per minute. In not a single instance did the respiratory volume during the anoxia produced by breathing 4.2, 5.2 or 6.0 per cent oxygen drop below the level observed in the preliminary control period.

A second striking feature is the wide range in the respiratory volumes. This is a well-known characteristic of the response of healthy men to anoxia. It accounts in part for the variability in their ceiling.

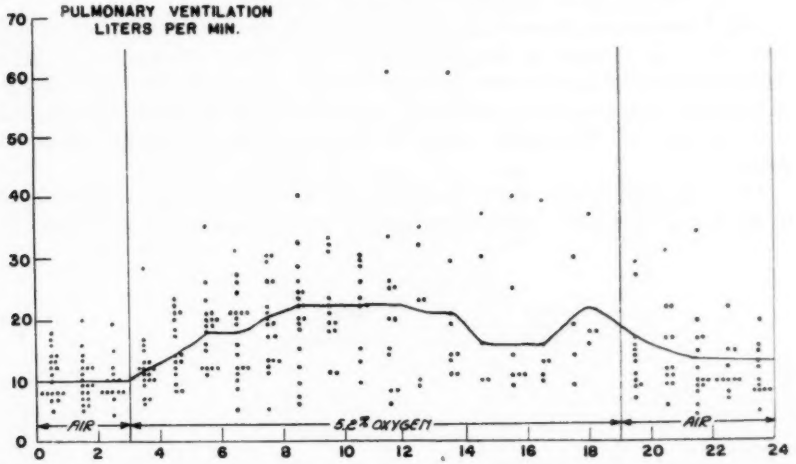

Fig. 2. Pulmonary ventilation before, during, and after breathing 5.2 per cent oxygen.

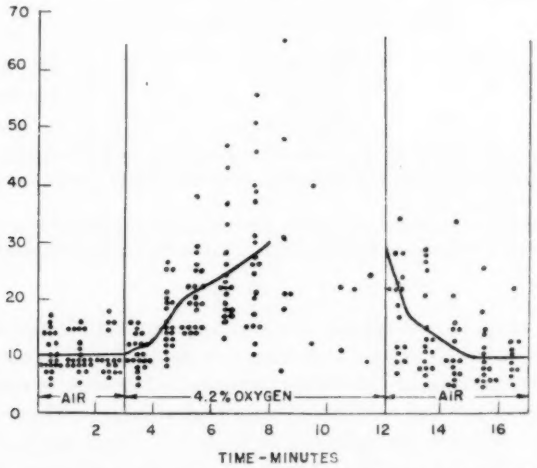

Fig. 3. Pulmonary ventilation before, during, and after breathing 4.2 per cent oxygen.

The third and most striking feature is the slow decline towards normal breathing in recovery. It is taught by physiologists that a period of overventilation is followed by a period of diminished ventilation or of apnea. No such decline was noted during the first five minutes of recovery in these patients. Following sudden access to air of normal composition, breathing usually continued above

the initial level for from 1 to 5 minutes and in no instance dropped to an alarmingly low level. This matter will be discussed below. In those cases where a shift was made from low oxygen to 14 per cent oxygen the respiratory response was the same as when the return was made to air.

Any considerable increase in respiratory volume during anoxia was accompanied by an increase in respiratory rate and depth. Schizophrenic patients characteristically have a rate above the range of most healthy individuals but in extreme anoxia the rate is increased even more—up to 50 times per minute in some instances. The mean values for three degrees of anoxia are shown in figure 4.

The composition of expired air indicates that a steady state was not attained in these experiments. The percentage of CO_2 in expired air continued to de-

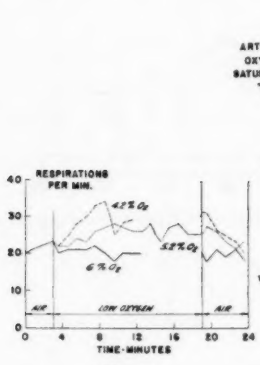

Fig. 4

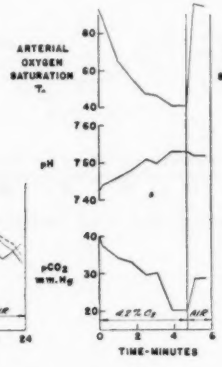

Fig. 5

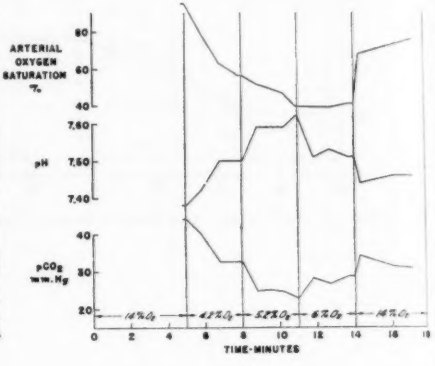

Fig. 6

Fig. 4. Respiratory rates before, during, and after periods of anoxia.

Fig. 5. Properties of arterial blood during and immediately after a five-minute exposure to 4.2 per cent oxygen.

Fig. 6. Properties of arterial blood while breathing atmospheres of various oxygen contents. The results indicate the interdependence of arterial oxygen, carbon dioxide, and degree of alkalosis.

cline. The decline in percentage of oxygen in expired air was abrupt at first and then more slow. A typical experiment is summarized in table 2.

Respiratory regulation at the onset of unconsciousness is revealed by the composition of expired air and respiratory rate and volume. These data, obtained in 20 tests on 7 subjects are collected in table 3. These data support the evidence given in figures 1 to 3 that the onset of unconsciousness in deep anoxia is not associated with respiratory arrest. Without exception the respiratory minute volume and the respiratory rate are high even though in some instances the gas expired contained less than 3.5 per cent oxygen.

Arterial blood was drawn during the height of the anoxia. Its analysis revealed oxygen saturations often as low as 50 per cent and sometimes below 40 per cent. Associated with the increased pulmonary ventilation there was ob-

TABLE 2

Time course of respiratory changes during inspiration of 4.2 per cent oxygen

Subject C. W. A., July 29, 1941

TIME	COMMENTS	COMPOSITION OF EXPIRED AIR		VENTILATION AT 760 MM. Hg 37°C.	RESPIRATORY RATE
		O_2	CO_2		
min.		*per cent*	*per cent*	*l./min.*	
0	Observations begun				
4	Breathing air	17.83	2.91	7.4	18
5	4.2% O_2 begun				
6		3.22	5.06	14.8	22
7		2.13	4.14	12.3	22
8		2.77	3.61	14.8	19
9	Unconscious	2.55	3.59	22.9	29

TABLE 3

*Gas samples obtained at the onset of unconsciousness**

DATE	SUBJECT	OXYGEN IN GAS INSPIRED	COMPOSITION OF GAS EXPIRED		PULMONARY VENTILATION AT 37°C., 760 MM. Hg.	RESPIRATORY RATE
			CO_2	O_2		
1941		*per cent*	*per cent*	*per cent*	*l./min.*	
July 23	B. H. E.	4.2	2.44	3.38	16.5	27
28		4.2	2.25	3.21	10.2	33
Sept. 5		4.2	2.07	4.79	19.6	21
10		5.2	1.67	3.80	25.4	35
15		6.0	1.36	4.51	11.2	40
July 22	C. J. G.	5.2	0.92	4.74	18.8	26
24		4.2	2.10	3.81	18.0	30
Sept. 9		4.2	1.68	4.22	21.7	38
11		4.2	2.13	3.45	18.2	36
July 22	S. J. A.	5.2	2.50	3.94	31.4	39
24		4.2	2.02	3.63	32.7	42
29		4.2	2.63	3.51	22.0	15
Sept. 16		4.2	2.59	4.38	47.5	37
July 17	C. W. A.	5.2	2.76	4.09	15.4	19
24		4.2	2.79	3.42	27.9	29
Sept. 9		4.2	2.95	3.07	23.3	19
July 23	D. F. W.	4.2	2.68	3.58	36.5	33
July 24	Q. B. J.	4.2	2.74	3.97	23.9	32
Sept. 9		4.2	2.50	3.99	26.8	31
Sept. 16	C. J. H.	4.2	2.95	3.35	21.0	19

* The gas samples were collected during a 30-second period. Eight were obtained just prior to unconsciousness and 12 extended into the period of unconsciousness.

served in the blood a decreased content of carbon dioxide and an increase in alkalinity, the pH often reaching 7.6. Some typical data are summarised in table 4, and the course of a typical run in which 4.2 per cent oxygen was breathed for 5 minutes is shown in figure 5.

In one type of test the patient was supplied with 14 per cent oxygen and then progressively with 4.2, 5.2 and 6.0 per cent oxygen each for 3 minutes. A needle was left in the artery and by changing syringes, enough samples were obtained to indicate the adaptive changes in the blood. These are presented in figure 6. The climax was reached at the end of the three-minute period on 5.2 per cent oxygen. Arterial saturation was then down to 40 per cent, the pH had risen to 7.6 and the pCO_2 had dropped to 23 mm. Hg. During the 3 minutes on the 6.0 per cent mixture the oxygen saturation remained constant, but the pH declined and the pCO_2 increased. The first stage of recovery was accomplished with 14 per cent oxygen. This was uneventful.

TABLE 4

Composition of arterial blood in the deepest observed anoxia

DATE	SUBJECT	EXPOSURE		TOTAL CO_2 CONTENT	CO_2 COMBINING CAPACITY	pCO_2	pH_g	O_2 SATURATION
1941		*min.*	*per cent O_2*	*vols. per cent*	*vols. per cent*	*mm. Hg*		*per cent*
July 16	B. H. E.	8.8	5.2	43.0	47.1	26.7	7.56	46
24	C. J. G.	11.5	4.2	41.9	45.9	24.2	7.58	43
22	S. J. A.	10.1	5.2	40.0	44.0	23.8	7.57	35
29	C. W. A.	8.9	4.2	37.9	48.0	20.2	7.53	41
21	D. F. W.	15.9	5.2	38.7	44.8	24.7	7.55	74
23	H. C. N.	7.2	4.2	41.5	42.2	31.3	7.45	46
21	M. D. R.	19.9	5.2	45.6	48.8	28.5	7.55	59
23	P. F.	7.4	4.2	43.4	43.6	32.5	7.45	51
22	Q. B. J.	10.0	5.2	46.8	49.3	29.1	7.51	50
17	C. J. H.	14.5	5.2	41.8	44.5	27.5	7.54	45

Clinical observations on the response of patients to 4.2 per cent oxygen are in brief as follows:

Cyanosis becomes quite noticeable in approximately 2 minutes; the ears are extraordinarily responsive. The hands get cold and clammy. At about the same time the patients seem to become sleepy. There is an obvious increase in the rate and depth of breathing. Perspiration becomes evident, especially in the axillae and the general thoracic areas.

Breathing becomes more difficult—sometimes gasping in nature. At this point the subjects frequently exhibit restless, unco-ordinated muscular movements, such as twitching of the mouth, chin, fingers, head, and even entire limbs. Saliva and mucus may accumulate and exaggerate the respiratory difficulties.

Sometimes the patients appear to hang on the verge of unconsciousness for several minutes. Their responses to commands become poor and answers to questions regarding date, age and birthday, that had been previously answered correctly, are frequently wrong. Repeating a question several times may elicit

a correct response even at the borderline of unconsciousness. At this point a few of the patients attempted to pull off their masks.

Unconsciousness, as evidenced by failure to respond to simple commands and the disappearance of the wink reflex, and in a few cases of the corneal reflex, comes on rather suddenly. In most cases this is so rapid as to give no preliminary warning. During the period of unconsciousness some become quite rigid with clonic movements. In two cases we observed spasmodic convulsions lasting respectively 15 and 60 seconds. These patients quieted down as the period of unconsciousness continued.

One patient commonly showed extensive and intensive muscular activity with rapid pulse and respiration before the anoxia was induced. After two minutes' exposure to low oxygen, he quieted down and remained so for some 5 to 10 minutes following completion of the test. In several other cases we also noted lessening of activity in the 5-minute recovery period. This may be associated with relief from the anxiety state into which the patient was thrown by the test.

On being returned to air or to 14 per cent oxygen, recovery is remarkably rapid, usually being well advanced within fifteen seconds. The ability to respond adequately to questions and commands is also restored rapidly. Cyanosis usually disappears within one minute. All effects are temporary in nature and leave the patient with neither ill effects nor recollections of the event.

Discussion. The therapeutic significance of these data will be presented in more detail elsewhere. It will suffice to say here that the mental condition of the patients was not changed. They were not benefitted by the procedures used nor, on the other hand, was there any evidence of damage to the central nervous system.

These patients revealed an unexpectedly high resistance to low oxygen mixtures and it seemed possible that loss of consciousness might occur at a higher level of arterial saturation if they had been in the erect or semi-erect posture. A few tests were made of this possibility by having the patient either standing or suspended in a harness such as forms a part of a parachute. The results were not different. This implies that in the stress of extreme anoxia not only the respiratory responses, but also the circulatory responses are strong and sustained; otherwise the cerebral circulation would reflect the changes in posture: consciousness would be lost earlier in the erect than in the reclining position.

These findings possess military significance because of their bearing on parachute escape. They indicate that flight personnel will not suffer permanent ill effects from oxygen lack if an escape is made at an altitude equivalent to a gas mixture containing 4.2 per cent oxygen.

The conversion of the percentages of oxygen used into equivalent altitudes is not simple. It cannot be accomplished by assuming that a given reduction in air pressure corresponds to a proportionate reduction in percentage of oxygen, the total pressure remaining constant, although this mistaken idea has persisted in the literature since the last war (4, 5). As a result of this error unreliable deductions have been drawn from observations of anoxia induced by air-nitrogen

mixtures or by a re-breather of the closed circuit type in which carbon dioxide is absorbed.

The usual method of calculation is based on the assumption that a given partial pressure of oxygen produces like physiological effects regardless of the total pressure. Thus it is commonly stated that 10.5 per cent oxygen at 760 mm. Hg is equivalent to 21 per cent oxygen at 380 mm. Hg. If the diluting effects of water vapor and of carbon dioxide are taken into account, it appears that 10.5 per cent oxygen is actually equivalent to a higher barometric pressure than 380 mm. Hg.

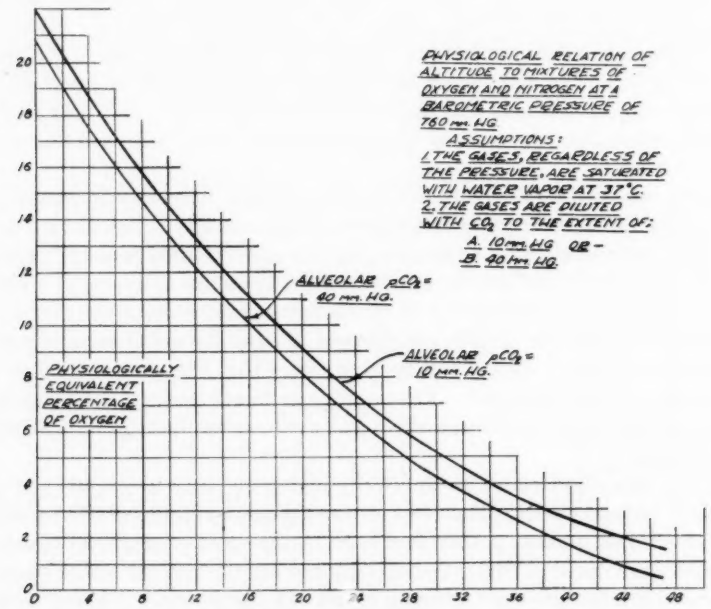

Fig. 7. Physiological relation of altitude to mixtures of oxygen and nitrogen inspired at sea level. Altitude is in thousands of feet.

A sample calculation is as follows:

It is assumed that the air as it is drawn into the lungs is warmed to 37°, saturated with water vapor up to pH_2O of 47 mm. Hg and admixed with enough carbon dioxide to yield a partial pressure of 40 mm. Hg. Hence, without allowing for the uptake of oxygen by the lungs, the partial pressures of oxygen (pO_2) in the two instances will be:

$$pO_2 = (760-47-40)0.105 = 71.7$$
$$= (380-47-40)0.21 = 61.5$$

It follows as will be seen below, that 10.5 per cent oxygen at ground level is equivalent to about 17,000 feet rather than 18,000 feet as is commonly stated. One consideration has not been taken into account in the foregoing calculation:

as carbon dioxide is being added, oxygen is being removed. The state of gas exchange in the lungs is one of dynamic equilibrium complicated by *a*, the constant movement of oxygen into the blood and of carbon dioxide out of the blood; *b*, by the rhythmic movement of air into and out of the lungs, and *c*, by the varying degrees of admixture of freshly inspired air with air in the depths of the lungs. As a first approximation, the method of calculation employed gives results in accord with empirical observations.

Equivalent altitudes have been calculated for two partial pressures of carbon dioxide. The results are shown graphically in the accompanying figure. If one accepts an intermediate pCO_2 of 30 mm. Hg as being characteristic of the tests made in this investigation, the altitudes may be set down as follows:

OXYGEN IN INSPIRED GAS	EQUIVALENT ALTITUDE
per cent	*feet*
4.2	31,000
5.2	28,000
6.0	26,000

These results have a military interest because of their bearing on the technic of parachute escape. In the absence of an accessory oxygen supply should one pull the rip cord at once if it is necessary to bail out at 31,000 feet? Romberg (6) felt that parachute descent from 36,000 feet, simulated in a low pressure chamber, was deleterious, being accompanied in all cases by collapse. He recommended a free fall until "safe" altitudes of 15,000 to 18,000 feet were reached. Since a man with an opened parachute will fall from 31,000 feet to 25,000 feet in about three minutes the tests reported herein indicate that the anoxia experienced while breathing air within this altitude range would have no harmful effects. Many individuals would not lose consciousness, and all those who do should fully recover consciousness long before ground level is reached.

SUMMARY

Schizophrenic patients have been subjected to severe anoxia over a period of several minutes either up to the point of unconsciousness or in some cases extending into unconsciousness. The following conclusions are drawn:

Anoxia of severe degree produces no beneficial effects on the mental condition of this class of psychotic patients.

Anoxia severe enough to produce brief periods of unconsciousness has no lasting harmful effects on the central nervous system.

Respiratory stimulation by anoxia is strong and sustained even during unconsciousness.

Inferentially, circulatory function is also well sustained.

There is a remarkably rapid return to normal when either air or 14 per cent oxygen is supplied.

A mixture of 4.2 per cent oxygen with nitrogen is equivalent physiologically to an altitude of about 31,000 feet.

It should be possible to descend with an opened parachute from 31,000 feet altitude without oxygen equipment with no ill effects from anoxia.

REFERENCES

(1) SAKEL, M. Nervous and mental disease. Monograph 62. Nervous and Mental Disease Company, Washington, D. C., 1938.
(2) HIMWICH, H. E., F. A. D. ALEXANDER AND B. LIPETS. Proc. Soc. Exper. Biol. Med. **39:** 367, 1938.
(3) CORWIN, W., D. B. DILL AND S. M. HORVATH. In press.
(4) Air Service Medical. War Department Air Service Division of Military Aeronautics, Washington. Government Printing Office. 1919, p. 166.
(5) VAN LIERE, E. J. Anoxia. Its effect on the body. Univ. of Chicago Press, 1942, p. 17.
(6) ROMBERG, H. W. Luftwissen **8:** 310, 1941.

AGE AND THE CALORIGENIC RESPONSE TO SUBCUTANEOUSLY ADMINISTERED ADRENALIN IN THE RAT

IVAN L. BUNNELL AND F. R. GRIFFITH, JR.

From the Department of Physiology, University of Buffalo, Buffalo, N. Y.

Received for publication November 5, 1942

Although the calorigenic response to subcutaneously administered adrenalin has been extensively studied in animals and man there is little to be found in regard to it in connection with the species most commonly used in metabolic work, the albino rat (Cori and Cori, 1928; Carr et al., 1934); and such information as there is describes only the total, average effect over $2\frac{1}{2}$ to 3 hour periods, with no information as to the time-course of the response.

The work to be described here was designed to fill this gap. In the course of it, evidence appeared that the character of the response altered significantly with age; this was therefore explored systematically with groups of animals from 2 to 28 months old.

METHODS. The animals used were male albino rats of our colony, a hardy, fertile, normal-growing Wistar strain inbred for generations and quite free from organic disease and internal or external parasites. They were reared and maintained on a diet of Purina Dog Chow in a room the temperature of which fluctuated only within the narrow range of 21 to 25°C.

Measurement of the respiratory metabolism was made with the apparatus described by Schwabe and Griffith (1938), modified for the CO_2 determination as described by Kingdon et al., (1942).

This apparatus is especially useful for the purpose here in mind since it provides continuous record of O_2 consumption and allows minute-to-minute measurement of CO_2 production. These are both indispensable for determination of the respiratory metabolism within short intervals and particularly for recognition and estimation of the values characteristic of the relatively short periods of quiescence on the part of the animal immediately after introduction into the chamber of the apparatus following the disturbing effect of an injection, and especially the injection of adrenalin.

In addition to initial, thorough calibration, the apparatus was subjected to frequent flame checks, by burning a tiny jet of illuminating gas at rates simulating the O_2 consumption and CO_2 production of the rat, after the manner of Bunnell and Griffith (1940).

During an experimental run the animal chamber of the apparatus was always at 28 to 29°C., the point of "thermic neutrality" as described by Benedict and MacLeod (1929).

DETERMINATIONS. A. BASAL. At 4 o'clock in the afternoon preceding a determination food was removed from the animal cage; the animal was then brought to the laboratory 17 to 18 hours later at 9 or 10 o'clock the next morning, placed in the chamber of the apparatus, and allowed as much time as necessary ($\frac{1}{2}$–$\frac{3}{4}$ of an hour) to settle down to a rigorously basal condition.

Following this basal determination the animal was removed from the apparatus and treated either as a control or with an injection of adrenalin as follows.

B. CONTROLS. *1. Handling.* After the basal determination, as just described, the animals to be used for this purpose, five in number, were removed from the apparatus and handled as if to receive an injection. They were then replaced and determination of metabolic rate made as frequently as possible for the next four hours. Although the record consists only of data derived during intervals of no overt activity, these, especially during the first half-hour or so, after return to the chamber, were of short duration and not likely to have been preceded by a sufficiently long rest period to be considered strictly basal. But since, as will be seen, it was equally or even more impossible to secure rigorously basal determinations within the first hour or so after injection of adrenalin, this method of procedure was all the more necessary for proper evaluation of the immediate, specific action of adrenalin itself. After this preliminary period following return to the chamber, and whether merely handled or injected, the animal settled down sufficiently so that most of the determinations from then on approximate basal values.

2. Saline injection. Following the preliminary basal determination the animals used for this purpose, three in number, were removed from the apparatus and injected subcutaneously with 0.9 per cent NaCl in amount equal to that serving as vehicle for the injected adrenalin as described below. They were then immediately replaced in the chamber of the apparatus and determinations made as frequently as possible and whenever there was no overt activity during the following hour. As mentioned under the previous heading these, for the most part, could not be strictly basal, but corresponded even more closely to those obtainable immediately after injection of adrenalin and are, therefore, particularly useful in evaluation of its effect.

C. ADRENALIN INJECTION. Again, this followed a preliminary basal determination, the animals, thirty-three in number, then being removed from, injected and returned to the apparatus with as little delay as possible and the metabolic rate followed as continuously as could be done with avoidance of periods of active movement, for the following four hours.

For the first hour or so after this injection the animal was particularly unquiet with a type of restlessness qualitatively different from the mere occasional bodily movement shown by the controls and characterized by panting, fine tremor (Choi, 1928; Hartman et al., 1927, 1928, 1929) and lying on the back. These and other activities subsided toward the end of the first hour after injection so that from then on the determinations again approach basal values. Such, however, are impossible to obtain during the early part of the action of adrenalin in the dosage employed here.

Following precedent (Cori and Cori; Carr et al.), the dose used was 0.02 mgm. per 100 grams body weight. This was injected with the necessary amount of 0.9 per cent NaCl (0.1 cc. of 1:1000 Parke-Davis adrenalin chloride solution was diluted to 1 cc. with salt solution and the requisite amount of this used for injection) subcutaneously on the medial aspect of the thigh.

RESULTS. *Controls.* Figures 2 and 3 show the extent to which measurement of the respiratory metabolism is affected by handling, control saline injection and the effort to secure determinations immediately after placement of the animal in the chamber of the apparatus and before there has been time for it to quiet down to a truly basal condition.

Oxygen consumption (fig. 2) is apparently unaffected to any significant degree by these experimental procedures. Considering that the beginning of these records coincides with about the 18th or 19th hour of fasting, the continuous slight decline of the record for the handled rats for the 4-hour period shown in the graph is understandable as the effect of prolonged fasting time. The absence of any noticeable elevation of rate above basal during the first hour of the record, when the animals were most restless shows that as long as measurements are confined to

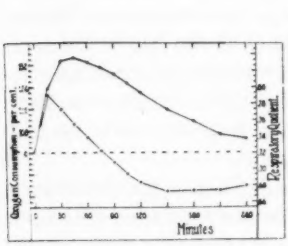

Fig. 1

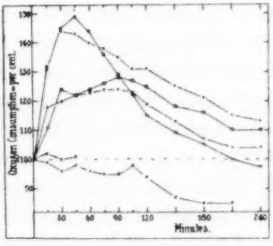

Fig. 2

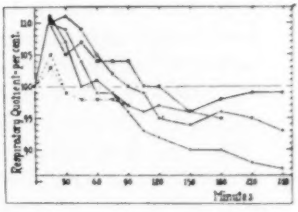

Fig. 3

Fig. 1. Upper curve, oxygen consumption (per cent of the preliminary basal value) and lower curve, respiratory quotient (actual values) for four hours following subcutaneous injection of 0.02 mgm. per 100 grams body weight; average responses of 33 male albino rats varying in age from 2 to 28 months.

Fig. 2. Oxygen consumption (per cent of preliminary basal) of controls and following the injection of adrenalin (dose as in fig. 1) at different ages:

x —— x ——, controls. Upper curve, saline injected (3 animals); lower curve, handled only (5 animals).

○ —— ○ ——: 2–6 months of age (9 animals).

● —— ● ——: 12–18 months of age (8 animals).

+ —— + ——: 19–24 months of age (9 animals).

□ —— □ ——: 25–28 months of age (7 animals).

Fig. 3. Respiratory quotient (per cent of preliminary basal) of controls and following the injection of adrenalin (dose as in fig. 1) at different ages; the different curves as in figure 2.

the even, short periods in which there was no overt movement these are not significantly affected by activity of the degree here encountered though it immediately preceded the determination.

Respiratory quotient (fig. 3) is more noticeably affected, especially during the first half-hour after replacing the animal in the apparatus. During this time it is raised temporarily 3–5 per cent above the previously determined basal, indicating that the restlessness characteristic of this early period before the animal settles down sufficiently to make strictly basal determinations possible does have a carry-over effect on CO_2 output during such brief periods of inactivity as occur and are measurable.

Adrenalin: A. Average effect. Figure 1 describes the total average effect on oxygen consumption and respiratory quotient for four hours following the administration of adrenalin. Each curve is the average of 33 complete, four-hour determinations.

Oxygen consumption will be seen to reach a maximum, 34.5 per cent above the preliminary normal basal value, 45 minutes after injection. From then on there is almost uniform decline which does not quite reach the initial basal value at the end of the four hour period. The actual figures in percentage above normal, for each of the subsequent time intervals shown on the curve are: 60 min., 33.1; 75 min., 31.1; 90 min., 28.9; 105 min., 25.5; 120 min., 22.1; 150 min., 15.9; 180 min., 11.8; 210 min., 6.8; 240 min., 5.6. Actually, if as probably should be done, the four-hour control curve of figure 2 were made the basis of estimate throughout, rather than the initial preliminary basal determination, the increase due to adrenalin would be greater at all time intervals and the elevation above the expected normal rate (reduced by prolonged fasting time) at the end of the four-hour period, approximately 20, rather than 5 per cent. Evidently, then, adrenalin is either still being actively absorbed four hours after injection (Cannon, 1929; Cori, 1929) or the metabolic disturbances initiated by it (Cori, 1931) are still responsible for a maintained elevation of O_2 consumption.

How much of the initial, peak-rise in oxygen consumption is attributable to the restlessness caused by this dosage of adrenalin cannot be estimated with complete certainty. It cannot be stressed too strongly that basal values, in the accepted sense of determinations made while the animal is quiet and after it has been so for a sufficiently long preliminary period, are impossible to obtain for an hour or so after injection of the amount of adrenalin used here. One can either measure the total metabolic rate as has been done by others; or as has been done here, and with an apparatus which we believe is sensitive enough to do so with some accuracy, confine the measurement to those short intervals in which the animal is not actually moving about, although it has been doing so immediately before. The control curves of figure 2 indicate that this can apparently be done with some success in the case of normal animals; and that the amount of activity resulting from preliminary handling, control saline injection, and recent introduction into the apparatus does not prevent measurements confined to short, quiet intervals from approximating very closely the actual basal value. To this extent there can be confidence that the adrenalin effect as we have measured it is not largely attributable to overt activity. The fine muscular tremor and panting induced by this amount of adrenalin cannot, however, be evaluated by control experiments; indeed, as an apparently inevitable and integral part of the action of adrenalin (in this amount) it would probably be unwise to attempt the exclusion of their effect from measurement of the total metabolic response to adrenalin as we are interested in it here.

From the beginning of the second hour these disturbing factors progressively lessen and the determinations from then on approach more and more closely truly basal values.

The total increase in O_2 consumption for the 4-hour period, as obtained by

rough integration of the area beneath the curve of figure 1, amounts to 19.5 per cent. This agrees closely with the increase of approximately 17 per cent obtained by the Coris (1929) for the first 3 hours, but is considerably less than the 32 per cent increase reported by Carr et al. (1934) as the total increase for the first 2½ hours following injection. In fact, in both of these instances values higher than ours should be expected since they apply only to the earlier, more markedly increased part of the response and the methods used measured the total rate of metabolism and did not admit of any attempt to eliminate periods of overt bodily movement.

Respiratory quotient is also seen from figure 1 to increase sharply within the first 15 minutes following injection, from an average basal value of 0.72 to a maximum of 0.79. Thereafter it falls progressively, reaching normal again within 75 minutes, and continuing to decrease to a minimum of slightly below 0.68, which is held between the 150th and 210th minutes; within the final half-hour there is apparently the beginning of a return toward normal.

The initial peak increase apparently is due in considerably greater part in this case than in that of O_2 consumption to the experimental procedures of handling, injection and immediate introduction of the animal into the apparatus. As shown by the control curves of figure 3, these are associated with a definite increase (to 0.742 and 0.757, respectively) in the respiratory quotient during the initial half-hour. The difference, however, between the controls and adrenalin-injected animals is considerable and shows that most of the increase during this time and until the end of 75 minutes is due to adrenalin. What part of this is a specific effect on the nature of the foodstuffs burned and what is indirect and related to the acidosis (lactacidemia) and excitement, with increased pulmonary ventilation and "Auspumpung" (Griffith et al., 1939, 1940) cannot be completely decided from the evidence at hand. The latter, however, would seem to be especially implicated since the subsequent depression below normal almost entirely compensates for this initial rise; the total respiratory quotient for the entire four-hour period, 0.71, as obtained by rough integration of the curve of figure 1 is, as was previously noted, also, by the Coris, and Carr, et al., practically unchanged from the preliminary normal basal value.

Adrenalin. B. The effect of age on the response. As mentioned in the introduction, it seemed evident from some of the early experiments which were done on animals of quite different ages, that this was responsible for an alteration in the nature of the response. This was thereafter taken into consideration and the work extended to include animals from 2 to 28 months old. The results are presented in figures 2 and 3, for which purpose the total number of 33 animals has been arbitrarily divided into four groups of approximately equal number (2–6 months of age, 9 animals; 12–18 months, 8 animals; 19–24 months, 9 animals; 25–28 months, 7 animals) in order to secure average curves of comparable validity.

Oxygen consumption. It is apparent from figure 2 that the response of animals less than 18 months of age differs markedly during the first 1½ hours from that of those which are older. Both of the younger groups show a sharp and prompt increase to a maximum (45 and 49 per cent) within 30 or 45 minutes after injec-

tion. In those animals 19 months of age and older this peak is entirely absent, the O_2 consumption rising only slowly to a maximum (24 and 28 per cent) about 90 minutes after injection. At this time the increase is about the same in all age groups and hereafter the O_2 consumption returns toward normal at an approximately equal rate for all such differences as are shown by the different groups during this later portion of the response probably being due merely to insufficient evidence.

It seems improbable, however, that mere lack of data can be responsible for the apparently pronounced difference in the earlier part of the response, although more extensive evidence is probably needed even here to describe the difference with real accuracy. It would seem improbable, for example, that the transition from one type of response to the other should be as abrupt as these results indicate. More likely, an adequate amount of data month-by-month would provide evidence of a more gradual transition; indeed, something of the sort seems to be indicated even by these meagre data: the maximum peak-response is shown by the youngest group of rats; the next older group shows a slight reduction toward the type of response typical of the oldest groups.

The only explanation that suggests itself for this difference is a slower absorption of adrenalin from the subcutaneous depot in the older animals. Although this assumption does not seem too improbable in light of the known ageing of the vascular system (Cohn, 1939) we know of no direct evidence in favor of it.

Respiratory quotient is not affected in the same way as oxygen consumption, although here, too, age does seem to alter the response. It will be seen from figure 3 that the maximum peak increase is practically the same in all age groups, both as to magnitude (10–11 per cent) and time of occurrence (15–30 min.) after injection. What difference there is seems to lie in the length of time the respiratory quotient is elevated above normal; this is least for the youngest age group and greatest in the oldest. Although it is not easy to see how maximum intensity could be unaffected by varying rate of adrenalin absorption, a delay of this with age would account for the prolongation of the increased quotient and its delayed return to normal.

Any further speculation as to the cause of these apparently different results at different ages would be premature until the fact is better established by more abundant evidence. These data are only sufficient to arouse interest in the possibility that a difference does exist.

SUMMARY

Subcutaneous injection of adrenalin (0.02 mgm. per 100 grams body weight) in male albino rats, 2 to 28 months old, causes an average maximal increase in O_2 consumption of 34.5 per cent within 45 minutes after injection; thereafter there is gradual decline to a value still about 5 per cent above the preliminary basal figure four hours after injection. Respiratory quotient increases from an average basal value of 0.72 to 0.79 within 15 minutes after injection; from this maximum it declines to normal within 75 minutes and continues to decrease to approximately 0.67 which is reached within 150 minutes and is maintained with only

slight tendency toward recovery until the end of the four-hour period. The total average increase in O_2 consumption for the four-hour period is 19.5 per cent; the preliminary increase in respiratory quotient is so nearly balanced by the compensatory fall that the total value for the four-hour period is practically unchanged from the preliminary normal.

In the older rats the maximum increase in oxygen consumption is less and is delayed until 90 minutes after injection; the maximal increase in respiratory quotient is practically the same and is reached at about the same time in all age groups, but the return to normal seems significantly delayed in proportion to increasing age. A delay in the absorption of the subcutaneously injected adrenalin proportional to age is tentatively suggested as a possible cause of these effects.

The increase in respiratory metabolism during the first hour or so after injection of this standard dose of adrenalin is undoubtedly due in part to the restlessness which it causes. The values reported here are probably not greatly affected by overt bodily movement, but it is impossible to eliminate the effect of the persistent muscular tremor which is characteristic of this initial period of action of this amount of the hormone.

REFERENCES

(1) Benedict, F. G. and G. MacLeod. J. Nutrition **1:** 367, 1929.
(2) Bunnell, I. L. and F. R. Griffith, Jr. Proc. Soc. Exper. Biol. and Med. **44:** 509, 1940.
(3) Carr, C. J., J. E. Schmidt and W. Harne. J. Pharmacol. Exper. Therap. **51:** 151, 1934.
(4) Cannon, W. B. Science **70:** 500, 1929.
(5) Choi, Y. O. This Journal **83:** 413, 1928.
(6) Cohn, A. E. Problems of ageing. Edited by E. V. Cowdry, Chapter 7. The Williams & Wilkins Co., Baltimore, 1939.
(7) Cori, C. F. Science **70:** 355, 1929.
(8) Cori, C. F. Physiol. Reviews **11:** 211, 1931.
(9) Cori, C. F. and G. T. Cori. J. Biol. Chem. **79:** 309, 321, 343, 1928.
(10) Griffith, F. R., Jr., F. E. Emery and J. E. Lockwood. This Journal **128:** 281, 1940; **129:** 155, 1940; **130:** 197, 1940.
(11) Griffith, F. R., Jr., J. E. Lockwood and F. E. Emery. This Journal **127:** 419, 1939.
(12) Hartman, F. A., J. I. Evans, B. T. Malachowski and L. M. Mickalek. This Journal **85:** 99, 1928.
(13) Hartman, F. A., J. I. Evans and H. G. Walker. Science **65:** 236, 1927. This Journal **81:** 482, 1927; **85:** 91, 1928; **90:** 668, 1929.
(14) Kingdon, C. L., I. L. Bunnell and F. R. Griffith, Jr. This Journal **137:** 115, 1942.
(15) Schwabe, E. L. and F. R. Griffith, Jr. J. Nutrition **15:** 187, 1938.

THE EFFECT OF DIETARY COMPOSITION ON PANCREATIC ENZYMES

M. I. GROSSMAN, HARRY GREENGARD AND A. C. IVY

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

Received for publication October 31, 1942

The ability of the pancreas to alter its external secretion by increasing the output of any given enzyme or enzymes in relation to the predominance of any particular substrate in the diet is a question which has long been debated and never adequately decided. Many years ago it was stated without the submission of adequate evidence that the pancreatic enzymic secretory response to a single meal was adapted to the type of food (1)—e.g., a meat meal elicited a secretion higher in tryptic activity than a meal low in protein.

Today it is generally believed that variations in the principal pancreatic enzymes take place in a parallel manner; in other words, a rise or fall in any one enzyme is accompanied by a coincident change in each of the others. For example, cholinergic drugs elicit a concentrated secretion rich in enzymes, whereas secretin provokes the formation of a dilute juice of low enzyme content; but in any given animal the ratio of concentration of the individual enzymes is the same, regardless of the type of stimulus. Likewise, the pancreatic juice formed in response to various meals may vary in concentration, but not in the relative amounts of the several enzymes. This belief may be true; yet it is still possible that, for example, a diet high in protein after being fed for several days may call forth a secretion richer in trypsin.

The parallelism of enzyme concentrations in the course of acute experiments has been established for a number of species, including the dog (2–4), rabbit (5), cat (6), and human (7). However, the ratio of the concentrations of the various enzymes shows marked variation, both from animal to animal and in the same animal over considerable periods of time. It is not inconceivable that such changes are the result of adaptation to a pronounced and prolonged change in the diet.

This possibility has been examined and never adequately confirmed. Two reports from Pavlov's laboratory (8, 9) state that in pancreatic fistula dogs the protease content increased on a meat diet and subsequently fell off on a high carbohydrate regime. In each case data were apparently obtained on only one animal and the findings were never confirmed. Later investigators devoted their attention to determinations of active and inactive enzymes on the various diets, with inconclusive results (10). More recently the enzyme content of the duodenal drainage of human subjects on various diets has been found to vary in relation to the predominating type of foodstuff in the diet (11) with noticeable differences detectable within a few days.

That this problem has not been adequately settled in the past is due to a

number of causes, including *a*, the variability in secretion of pancreatic fistula dogs, which would require sufficient data for statistical analysis; *b*, the inadequate number of control animals and control periods; *c*, inaccurate dietary control; *d*, insufficient duration of experimental observation; *e*, inaccurate methods for enzyme analysis; and *f*, in the case of duodenal drainage fluid, the contaminated condition of the pancreatic juice.

A technique calculated to overcome these difficulties has been devised by us, which depends for its effectiveness on the principle that the concentration ratio of enzymes in pancreatic juice is the same as that existing in the gland which secretes it. The accuracy of this conception was established by appropriate analyses of pancreatic secretion and pancreatic tissue in a series of dogs, and thus the necessity of fistula animals was eliminated. A number of diets of varying compositions was then fed to white rats for a requisite interval, at the conclusion of which the enzyme content of the pancreatic tissue was determined.

EXPERIMENTAL. The methods for enzyme analysis were those successfully employed in this laboratory for some time. Lipase was determined by the method of Cherry and Crandall (12), amylase by the Willstätter (13) procedure as adapted by us (14), and trypsin by a colorimetric method devised after that of Northrop (15). The results are expressed in the tables below as follows: lipase in cubic centimeters of 0.05 N NaOH required to neutralize the fatty acids liberated from an olive oil emulsion substrate, amylase in milligrams of maltose liberated from a starch substrate, and trypsin in terms of milligrams of tyrosine liberated from a casein substrate.

The enzyme content of pancreatic tissue was determined on weighed amounts of the dried gland. The pancreas was removed immediately after death of the animal, macerated in a mortar, and suspended in cold (15°C.) acetone for 3 hours. The acetone was removed by centrifuging and the tissue resuspended in fresh acetone for 10 minutes at room temperature, collected by centrifuging, washed with dry ether, and finally air-dried, pulverized, and stored in stoppered glass containers. Weighed amounts of the powdered gland were suspended in 1/15 molar phosphate buffer at a pH of 7 for one hour at room temperature in the proportion of 0.2 or 0.3 cc. per milligram, the mixture filtered, and the enzyme content of an aliquot of the filtrate determined.

In the series of dogs employed for purposes of comparison of enzyme content in pancreatic secretion and in the gland itself, the procedure involved anesthetization of the animal with sodium pentobarbital, cannulation of the main pancreatic duct, and collection of the secretion after stimulation with an adequate amount of secretin. The pancreas was then excised and treated as indicated above.

One hundred sixty-two young white rats (100–150 grams) served as experimental animals for the study of adjustment of pancreatic enzymes to the type of food administered. The diets had the composition shown in tables 1 and 1A and were fed *ad libitum* for a period of 21 days, at the completion of which the animals were sacrificed by cervical fracture, and the pancreas promptly and completely dissected out, and treated as above for enzyme determinations.

Most of the animals were maintained on isocaloric diets (table 1A), in order to make certain that all essential components were ingested in amounts exceeding the minimum requirement, and thus avoid complications which might arise from any deficiency.

RESULTS. 1. *Comparison of enzyme content of pancreatic juice and of dried pancreas.* It was found in the ten animals (anesthetized dogs) tested that in

TABLE 1

Composition of high carbohydrate, high protein and high fat diets

	DIET A HIGH CHO	DIET B HIGH CHO	DIET C HIGH PROTEIN	DIET D HIGH PROTEIN	DIET E HIGH FAT
Casein	18	10	60	65	10
Dextrin	47	55	0	0	0
Sucrose	13	13	13	13	13
Salt mixture (Osborne-Mendel)	4	4	4	4	4
Lard	5	5	10	5	60
Agar	2	2	2	2	2
Fish liver oil	3	3	3	3	3
Yeast	8	8	8	8	8

TABLE 1A

Isocaloric diets

	DIET H BALANCED			DIET I HIGH CHO			DIET G HIGH PROTEIN			DIET F HIGH FAT		
	Wt.	Cal.*	Cal.	Wt.	Cal.	Cal.	Wt.	Cal.	Cal.	Wt.	Cal.	Cal.
	per cent		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*
Casein	18	72	15	15	60	15	67	268	65	25	100	15
Starch	47	188	38	67	268	65	15	60	15	0	0	0
Salt	4	0	0	4	0	0	4	0	0	4	0	0
Cellulose	2	0	0	2	0	0	2	0	0	2	0	0
Lard	18	162	33	3	27	6	3	27	6	54	486	72
Yeast	8	40	8	6.5	33	8	6.5	33	8	11	55	8
Fish liver oil	3	27	5	2.5	23	5	2.5	23	5	4	36	5
Totals	100	489	99	100	411	99	100	411	99	100	677	100

* Calories.

each case there was a parallelism between the concentration of the three chief pancreatic enzymes present in the gland itself and in the juice secreted by it. The results in detail are listed in table 2.

2. *Enzyme composition of pancreatic tissue of rats on constant diets.* When the enzyme values for the various diets are compared with those of diet H, the balanced diet, a number of differences are observed. The series of animals fed the high carbohydrate diet (diets A, B and I) showed a marked predominance of

amylase in the pancreatic tissue. There was a repression of trypsin, and the lipase value was unchanged. Likewise, there was an increased trypsin content in the rats fed the high protein diet (diets C, D and G). On the high fat regime (diets E and F) there was repression of amylase, while the lipase and trypsin

TABLE 2

Enzyme composition of 100 mgm. of pancreatic extract and 0.02 cc. of pancreatic juice in the same animal

DOG NO.	SOURCE	AMYLASE		TRYPSIN		LIPASE	
		Maltose	Ratio extract to juice	Tyrosine	Ratio extract to juice	NaOH	Ratio extract to juice
		mgm.		*mgm.*		*cc.*	
1	Extract	420	0.44	9.32	0.47	156	0.44
	Juice	950		19.9		355	
2	Extract	390	0.58	6.32	0.78	132	0.68
	Juice	675		8.15		195	
3	Extract	434	0.71	13.6	0.68	202	0.70
	Juice	615		20.1		290	
4	Extract	360	0.47	9.88	0.48	168	0.48
	Juice	770		20.4		350	
5	Extract	292	0.53	11.6	0.49	126	0.52
	Juice	550		23.9		240	
6	Extract	286	0.45	10.3	0.40	242	0.49
	Juice	642		25.8		494	
7	Extract	362	0.74	12.8	0.82	298	0.84
	Juice	488		15.6		354	
8	Extract	435	0.76	21.4	0.79	308	0.81
	Juice	572		27.1		380	
9	Extract	392	0.82	17.4	0.81	281	0.84
	Juice	478		21.2		334	
10	Extract	414	0.69	29.8	0.61	386	0.58
	Juice	600		48.8		665	

values remained essentially unaltered. All enzymes were repressed on a high fat diet containing 10 per cent protein (diet E); the 24 animals in this series had fatty livers and small atrophic pancreases. Addition of 1 per cent of choline to the diet resulted in an increase in all the pancreatic enzymes to approximately the same degree, and a normal histological picture of the liver and pancreas.

The detailed data for all animals and the average values are listed in table 3.

DISCUSSION. It is apparent on the basis of the data submitted that the type of diet ingested influences the enzyme make-up of the pancreas and its secretion. Thus the 39 rats on a high carbohydrate diet developed very high amylase values, with a repression of trypsin and essentially unchanged lipase. The series on a high protein intake had a high trypsin content of the pancreas; the lipase values were also highest on this regime, while the amylase was repressed. In the case of the high fat diet there was a repression of amylase, with essentially unaltered values of trypsin and lipase. The significance of the changes in composition was demonstrated by statistical analysis (table 4).

The most marked responses to dietary modifications are elevated amylase and depressed trypsin values in the case of a high carbohydrate diet, elevated

TABLE 3

Average enzyme content of pancreatic tissue of rats

NO. OF RATS	RAT NUMBERS	SERIES	DIET	DESCRIPTION	AMYLASE		TRYPSIN		LIPASE	
					Range	Ave.	Range	Ave.	Range	Ave.
4	1–4	I	A	High CHO	370–380	376	4.4– 8.0	5.5	74–94	86
10	13–22	II	B	High CHO	291–465	336	0.6– 33.0	9.2	309–519	406
25	118–142	IV	I	High CHO	546–1140	689	1.5– 17.4	9.1	216–423	333
4	5–8	I	C	High protein	152–304	225	4.4– 49.6	20.8	100–154	128
10	23–32	II	D	High protein	219–278	252	26.7– 68.4	48.2	360–660	556
25	68–92	IV	G	High protein	165–540	398	16.5–103.5	60.9	321–555	464
4	9–12	I	E	High fat	142–206	174	1.0– 29.6	9.4	64–88	75
10	33–42	II	E	High fat	9–282	147	8.1– 41.4	24.8	150–540	342
10	143–152	III	E	High fat	48–312	171	1.8– 12.9	5.8	51–123	83
10	153–162	III	E*	High fat plus 1% choline	108–462	309	9.6– 34.2	20.7	87–324	184
25	43–67	IV	F	High fat	60–264	170	3.0– 44.4	24.4	60–564	316
25	93–117	IV	H	Balanced	285–606	489	2.4– 73.5	20.3	192–495	358

E* = Diet E plus 1% choline.

trypsin on a high protein regime, and depressed amylase on a high fat intake. No marked alterations in lipase occurred on any of the diets, save for a definite increase when ½ of the diet was supplied by protein. These experiments, therefore, bear out in part the long-standing hypothesis that the composition of the pancreatic juice is governed by the type of foodstuffs ingested. The mechanism whereby this takes place is at present obscure, and its explanation must await an elucidation of the process of formation of the pancreatic enzymes. Whether this takes place on a metabolic, neurogenic, or other basis is at present unknown. The situation is somewhat analogous to that of some bacteria, which are known to produce certain enzymes in response to the predominance of a given substrate in the diet (16).

The animals on the high fat-low protein diet (diet E) manifested a depression of all enzymes in their pancreatic tissue. These animals remained in good

condition throughout the experiment, except for a shaggy and greasy appearance of the coat of hair. On autopsy the pancreas was small and atrophic, with indications of parenchymatous degeneration in the acinar tissue, while the islet tissue was normal in appearance, and the liver was very fatty. Addition of 1 per cent of choline to this diet (diet E) resulted in a uniform increase in all the enzymes determined (table 3). On this regime the liver and pancreas showed no histological changes.

As a matter of physiological economy, it might be expected that the enzyme output of a digestive gland should be subject to modification in accordance with

TABLE 4

Statistical analysis of enzyme data on rat 43-142, indicating significance of values observed

DIET	MEAN	STANDARD ERROR OF MEAN	DIFFERENCE OF THE MEANS	STANDARD ERROR OF DIFFERENCE OF MEANS	CRITICAL RATIO "C"	PROBABILITY OF OCCURRENCE OF A DEVIATION AS GREAT AS DESIGNATED "C"
			Amylase			
CHO	689	±23.3	200	±28.9	6.9	0.26×10^{-11} %
Bal.	489	±17.4	91	±31.9	2.9	0.373%
Prot.	398	±26.7	128	±28.9	4.4	0.003%
Fat	170	±11.0				
			Trypsin			
Prot.	60.9	±4.5	36.5	±5.1	7.2	$.26 \times 10^{-11}$%
Fat	24.4	±2.4	4.1*	±4.3	0.95	33%
Bal.	20.3	±3.6	11.2	±3.7	3.0	0.27%
CHO	9.1	±0.99				
			Lipase			
Prot.	464	±11.6	106	±18.2	5.8	2×10^{-8}%
Bal.	358	±14.0	25*	±16.8	1.5	13%
CHO	333	±9.3	17*	±27.3	0.6	50%
Fat	316	±25.6				

* These differences not significant.

the predominating foodstuffs present in the diet, in order that the organism may utilize its available materials with a maximum of efficiency. Such changes in composition as are brought about by any given dietary regime undoubtedly occur in the gland itself, and apparently the external secretion of the pancreas represents an aqueous extract of the organic material in the acinar tissue. This is borne out by the parallelism in enzyme content of the secreted juice and the dried pancreas in the ten dogs studied; and it is on the validity of this assumption that the accuracy of the interpretation of the data reported is based.

SUMMARY AND CONCLUSIONS

1. In rats maintained for three weeks on a constant diet, an adaptation of the composition of chief pancreatic enzymes to the predominant constituent of the

diet was noted. Thus on a high carbohydrate diet there was a pronounced increase in amylase, together with a decrease in trypsin. A high protein diet resulted in greatly increased trypsin content and a less extensive, but definite, increase in lipase. On a high fat diet there were essentially no alterations in lipase or trypsin.

2. A diet which is high in fat and low in protein causes a repression of all pancreatic enzyme formation. The addition of 1 per cent choline to such a diet increases uniformly the content of all enzymes.

3. The enzyme content of pancreatic juice parallels that existing in pancreatic tissue.

REFERENCES

(1) Walther, A. A. Loc. cit. I. P. Pavlov. The work of the digestive glands. Charles Griffin & Co., London, 1910.
(2) Babkin, B. P. Trans. Milit. Med. Acad. St. Petersburg **9:** 93, 1904.
(3) Sawitsch, W. W. Zentralbl. ges. Physiol. Path. Stoffw. **4:** 1, 1909.
(4) Greengard et al. To be published.
(5) Baxter, S. G. Am. J. Digest. Dis. **2:** 108, 1935.
(6) Harper, A. A. and C. C. N. Vass. J. Physiol. **99:** 415, 1941.
(7) Wohlgemuth, I. Berliner Klin. Wchnschr. **2:** 47, 1907.
(8) Vasilev, B. N. Arch. d. Sci. Biol. **2:** 219, 1893.
(9) Jablonski, V. M. Arch. d. Sci. Biol. **4:** 377, 1895.
(10) Babkin, B. P. Die Aussere Sekretion der Verdauungsdrusen. Julius Springer, Berlin, 1928.
(11) Michelson, V. Arch. f. Verdauungskrankheiten **51:** 73, 1932.
(12) Cherry, I. S. and L. A. Crandall. This Journal **100:** 266, 1932.
(13) Willstatter, R., R. Waldschmidt-Leitz and E. Z. Hesse. Physiol. Chem. **126:** 143, 1923.
(14) Schmidt, C. R., H. Greengard and A. C. Ivy. Am. J. Digest. Dis. **1:** 618, 1934.
(15) Northrop, J. H. J. Gen. Physiol. **16:** 313, 1932.
(16) Dubos, R. J. Bact. Rev. **4:** 1, 1940.

THE EFFECT OF ATROPINE ON THE CORONARY BLOOD FLOW OF TRAINED DOGS WITH DENERVATED AND PARTIALLY DENERVATED HEARTS

HIRAM E. ESSEX, J. F. HERRICK, FRANK C. MANN AND EDWARD J. BALDES

From the Division of Experimental Medicine, Mayo Foundation, and Division of Physics and Biophysical Research, Mayo Clinic and Mayo Foundation, Rochester, Minnesota

Received for publication November 25, 1942

In previous studies (1, 2) from this laboratory it has been shown that intravenous injections of atropine sulfate result in prolonged increases of coronary blood flow and heart rate in trained dogs with innervated hearts. Similar results were obtained on animals under chloralosane anesthesia. In addition it was found that changes of the arterial blood pressure following the administration of atropine sulfate were insignificant; therefore the augmented coronary blood flow could not result from a change of blood pressure. The possibility that the increased heart rate might be at least partially responsible for the greater coronary blood flow was explored by Hausner and others (3) using the so-called denervated heart-lung preparation. In that study it was found that increases and decreases of heart rate produced by means of Hill's stimulator caused, respectively, increases and decreases of the coronary blood flow. The absence of significant changes of blood pressure following injections of atropine left the augmented heart rate and certain nervous influences as possibly responsible for the increased coronary flow. It was also necessary to consider the possibility that atropine affected directly the walls of the coronary blood vessels.

In another investigation we were observing the effect of exercise on the denervated and partially denervated heart of the trained dog (4). The observations reported here were made on the animals used in that study.

METHODS. In the present study the response of the heart rate and the coronary blood flow to injections of atropine sulfate (0.1 to 0.2 mgm. per kgm.) was observed in dogs after the following procedures: 1, removal of both sympathetic chains of ganglia from the eighth or ninth intercostal space including the stellate ganglion; 2, double cervical vagotomy, and 3, a combination of procedures 1 and 2. The operations were done employing sterile technic and with the animals under general anesthesia. The section of the left vagus was usually done under infiltration anesthesia with procaine hydrochloride. The coronary blood flow was measured by means of the thermostromuhr and the units were applied at varying intervals after the operative procedures indicated under 1, 2 and 3. An effort was made to make all observations before sufficient time had passed for regeneration of the nerves to have occurred. The blood pressure was recorded optically from a cannulated femoral or carotid artery.

RESULTS. Although a considerable series of observations was made the results were sufficiently uniformly positive that only a few representative experiments will be described in detail.

Effects of atropine after sympathetic ganglionectomy. In the absence of the cardiac sympathetic nerves, injections of atropine caused a marked increase of heart rate and an augmented coronary flow of 25 to 100 per cent. The effect of the drug was evident in twenty to thirty seconds after injection. The blood pressure was not elevated significantly in any of the experiments. In the experiment represented in figure 1 the maximal change of coronary flow was nearly 100 per cent. The heart rate rose from 70 to 200 beats each minute. The rectal temperature was 102.1°F. The mean blood pressure taken by means of a glass spoon manometer (5) was practically unchanged for three minutes, when a few fluctuations occurred in the next two minutes. Thereafter, however, the blood pressure remained relatively constant. The coronary blood

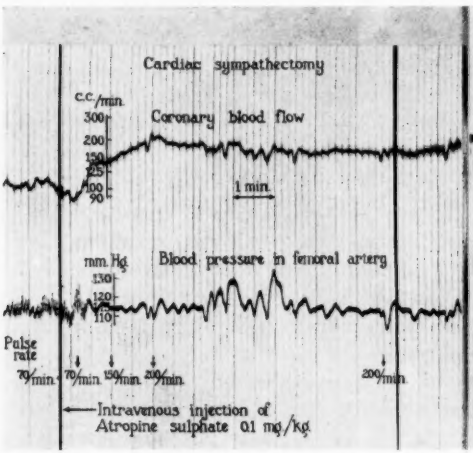

Fig. 1

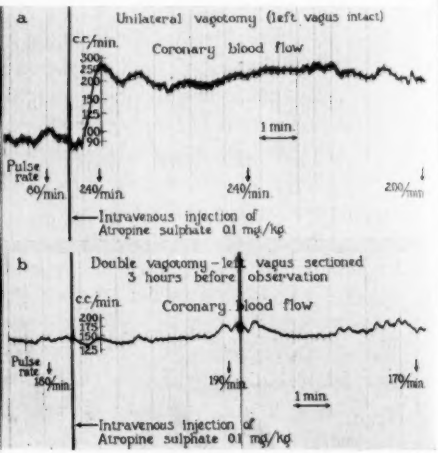

Fig. 2

Fig. 1. The effect of an intravenous injection of 0.1 mgm. per kgm. of atropine sulfate on the coronary blood flow, pulse rate and blood pressure of a dog after cardiac sympathectomy.

Fig. 2. Effect of an intravenous injection of 0.1 mgm. per kgm. of atropine sulfate on the coronary blood flow of a dog *a* before and *b* after the second vagus nerve was sectioned.

flow and heart rate of the dog in this experiment remained above the control values for more than an hour.

The thermostromuhr unit was applied one month and four days after the first sympathetic ganglionectomy. In the absence of the sympathetics but in the presence of the vagi, the heart accelerated to 200 beats each minute, which would indicate that it was under considerable vagal tone, removal of which by the effect of atropine allowed the heart marked acceleration. The acceleration was not the result of sympathetic tone since the sympathetics had been removed (fig. 1).

Effect of atropine after bilateral cervical vagotomy. Whether atropine affects

the coronary blood vessels directly, causing a vasodilatation, was considered and a series of experiments were done in which the vagus nerves were sectioned a month or so previously. In these animals the blood pressure, heart rate and coronary blood flow were unaffected by injections of atropine.

Observations were also made on another series of dogs in which the effect of the drug was observed before and a few hours after section of the remaining vagus nerve. In one experiment the control pulse rate before section of the remaining vagus nerve was 80 beats each minute and the coronary flow was about 100 cc. each minute. Three hours after section of the remaining vagus the control heart rate was about 180 each minute. The coronary blood flow was about 150 cc. each minute. An injection of atropine did not materially alter the control values (fig. 2). The results were the same following double cervical vagotomy whether or not the sympathetic nerves were present.

COMMENT. It appears that the cardiac acceleration resulting from vagal paralysis by injections of atropine sulfate was owing entirely to release of vagal influence or tone. The increased coronary blood flow was not the result of increases of the blood pressure because the blood pressure was not significantly altered. Since the injection of atropine had no effect on the blood pressure, heart rate or coronary blood flow in the totally denervated heart, it may be logically assumed that the increased coronary blood flow resulting from the injection of atropine into animals with innervated hearts and into animals with hearts supplied with only the vagus nerves is not owing to a direct dilator effect of atropine on the coronary vessels. In view of the fact that increases of heart rate alone have been shown to affect coronary flow decisively, one is led to the conviction that the acceleration of the heart, following administration of atropine in sufficient dosage, is responsible for the increased coronary blood flow. The mechanism by which this might be accomplished is, however, unknown.

SUMMARY AND CONCLUSIONS

In the present study the response of the coronary blood flow and heart rate to atropine sulfate has been observed after the following operative procedures: 1, right and left sympathetic ganglionectomy from the eighth or ninth intercostal space anteriorly including the stellate ganglion; 2, double vagotomy in the neck; 3, a combination of procedures 1 and 2. In the absence of the sympathetic nerves, as in procedure 1, atropine caused increases of 25 to 85 per cent in coronary flow and an increase in pulse rate of a similar magnitude. Atropine was without effect on the coronary blood flow, heart rate or blood pressure of vagotomized animals or animals with denervated hearts. It may be concluded that the increased coronary blood flow following injections of atropine is not owing to a direct effect of the drug on the wall of the blood vessel nor is it due to changes of blood pressure. The augmented coronary flow follows the inhibition of vagal tone and is associated with the resulting increased cardiac rate. When all of the evidence is considered it is difficult to escape the conclusion that the increased heart rate itself is responsible for the increased coronary blood

flow following administration of atropine but the mechanism by which it is accomplished is not apparent.

REFERENCES

(1) Essex, H. E., R. G. E. Wegria, J. F. Herrick and F. C. Mann. Am. Heart J. **19**: 554, 1940.
(2) Wegria, R., H. E. Essex, J. F. Herrick and F. C. Mann. Am. Heart J. **20**: 557, 1940.
(3) Hausner, E., H. E. Essex, J. F. Herrick and E. J. Baldes. This Journal **131**: 43, 1940.
(4) Essex, H. E., J. F. Herrick, E. J. Baldes and F. C. Mann. Am. J. Physiol. (In press).
(5) Kubicek, W. G., F. P. Sedgwick and M. B. Visscher. Rev. Scient. Instruments **12**: 101, 1941.